Hines
09/591447

- keyterms

FILE 'CAPLUS' ENTERED AT 12:40:53 ON 06 FEB 2002

L1 560 SEA FILE=CAPLUS ABB=ON PLU=ON SURA OR (SUR OR HTR)(W)A OR NIRB OR NIR B OR HTRA OR AROD OR AROE OR ARO(W)(D OR E)

L2 141 SEA FILE=CAPLUS ABB=ON PLU=ON L1(S)(MUTANT OR MUTAT? OR MUTAGEN? OR POLYMORPH? OR POLY MORPH?)

L15 59 SEA FILE=CAPLUS ABB=ON PLU=ON L2 AND (BACTERI## OR VIBRIO OR HAEMOPHILUS OR HEMOPHILUS OR NEISSER? OR YERSIN? OR BORDETELLA OR BRUCELLA)

L16 21 SEA FILE=CAPLUS ABB=ON PLU=ON L15 AND (VACCIN? OR IMMUNIS? OR IMMUNIZ?)

L16 ANSWER 1 OF 21 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:297553 CAPLUS
DOCUMENT NUMBER: 134:321599
TITLE: Cloning of Lawsonia genes htrA, ponA, hypC, lysS, ycfW, abc1, and omp100, their encoded proteins or peptides and therapeutic use in diagnosis and as **vaccine**
INVENTOR(S): Rosey, Everett Lee
PATENT ASSIGNEE(S): Pfizer Products Inc., USA
SOURCE: Eur. Pat. Appl., 80 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1094070	A2	20010425	EP 2000-309125	20001017
EP 1094070	A3	20020109		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001169787	A2	20010626	JP 2000-320736	20001020

PRIORITY APPLN. INFO.: US 1999-160922 P 19991022

AB The present invention relates generally to therapeutic compns. for the treatment and/or prophylaxis of intestinal disease conditions in pigs or other animals caused or exacerbated by Lawsonia intracellularis or similar or otherwise related microorganism, such as porcine proliferative enteropathy (PPE). In particular, the present invention provides novel genes htrA, ponA, hypC, lysS, ycfW, abc1, and omp100 derived from Lawsonia intracellularis genomic regions A and B. These genes encode sequence homologs to lysyl-tRNA synthetase (gene lysS), transmembrane or integral membrane protein (abc1), hydrogenase maturation protein (hypC), penicillin binding protein (ponA), and periplasmic serine protease protein (htrA) resp. The invention also relates to constructing these gene expression vector to produce recombinant protein using E. coli. Methods of expressing recombinant htrA and omp100 proteins in E. coli are also provided. The invention also provides the immunogenic peptides or proteins encoded by these genes that are particularly useful as an antigen in **vaccine** prepn. for conferring humoral immunity against Lawsonia intracellularis and related pathogens in animal hosts. The present invention is also directed to methods for the treatment and/or prophylaxis of such intestinal disease conditions and to diagnostic agents and procedures for detecting Lawsonia intracellularis or similar or otherwise related microorganisms.

L16 ANSWER 2 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:75665 CAPLUS
DOCUMENT NUMBER: 135:194118
TITLE: Properties of recombinant HtrA: An otitis media **vaccine** candidate antigen from non-typeable **Haemophilus** influenzae
AUTHOR(S): Cates, G. A.; Yang, Y.-P.; Klyushnichenko, V.; Oomen, R.; Loosmore, S. M.
CORPORATE SOURCE: Aventis Pasteur, Toronto, ON, Can.
SOURCE: Dev. Biol. (Basel., Switz.) (2000), 103(Physico-Chemical Procedures for the Characterization of Vaccines), 201-204
CODEN: DBEIAI; ISSN: 1424-6074
PUBLISHER: S. Karger AG
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Non-encapsulated or non-typeable H. influenzae (NTHi) is a major cause of middle ear infections in young children. HtrA has been identified as a **vaccine** candidate antigen from NTHi; therefore physicochem. characterization of this antigen is important for **vaccine** development. Recombinant NTHi HtrA has been expressed in E. coli and shown to have serine protease activity. Several **mutant**, recombinant **HtrA** proteins were expressed and purified to obtain suitable **vaccine** antigens lacking protease activity. Two mutants with alterations at the putative active site His91 and Ser197, designated H91A and S197A were examd. by circular dichroic spectropolarimetry (CD) to evaluate secondary structure. The S197A mutant had a more random secondary structure compared to wild-type rHtrA or H91A. It is likely that improper folding of S197A accounts for its lack of immunoprotective properties in a chinchilla model of otitis media.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 3 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:831674 CAPLUS
DOCUMENT NUMBER: 134:70048
TITLE: Susceptibility of calves to challenge with Salmonella typhimurium 4/74 and derivatives harbouring **mutations** in **htrA** or purE
AUTHOR(S): Villarreal-Ramos, Bernardo; Manser, Jacquie M.; Collins, Robert A.; Chance, Victoria; Eckersall, P. David; Jones, Phillip W.; Dougan, Gordon
CORPORATE SOURCE: Institute for Animal Health, Compton, RG20 7NN, UK
SOURCE: Microbiology (Reading, U. K.) (2000), 146(11), 2775-2783
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Salmonella typhimurium 4/74 is highly virulent for cattle after oral challenge, causing severe diarrhea, which is sometimes assocd. with systemic spread of the micro-organism. Although susceptible to oral challenge, groups of cattle were found to be relatively resistant to

s.c. challenge with this strain. The virulence of S. typhimurium 4/74 harbouring **mutations** in **htrA** and purE was also assessed in cattle. Although S. typhimurium 4/74 **htrA** and purE are attenuated following oral challenge in mice, cattle were highly susceptible to oral challenge with these **mutants** . As with the parent S. typhimurium 4/74 strain, cattle exhibited greater susceptibility to oral compared to s.c. challenge with S. typhimurium **htrA** and purE **mutants**. Following s.c. challenge with sublethal levels of S. typhimurium 4/74, calves produced significant levels of antibodies to S. typhimurium sol. ext. No correlation was detected between interferon gamma levels in sera and susceptibility to infection by any route. The concns. of the acute-phase-assocd. protein haptoglobin were increased in the sera of five of six cattle inoculated s.c., although increases in concn. were smaller in cattle inoculated orally.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 4 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:285371 CAPLUS
DOCUMENT NUMBER: 133:86644
TITLE: Investigation into the role of the serine protease HtrA in **Yersinia** pestis pathogenesis
AUTHOR(S): Williams, K.; Oyston, P. C. F.; Dorrell, N.; Li, S.-R.; Titball, R. W.; Wren, B. W.
CORPORATE SOURCE: Department of Infectious and Tropical Diseases, Pathogen Molecular Biology and Biochemistry Unit, London School of Hygiene and Tropical Medicine, London, UK
SOURCE: FEMS Microbiology Letters (2000), 186(2), 281-286
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The HtrA stress response protein has been shown to play a role in the virulence of a no. of pathogens. For some organisms, **htrA mutants** are attenuated in the animal model and can be used as live **vaccines**. A **Yersinia** pestis htrA ortholog was identified, cloned and sequenced, showing 86% and 87% similarity to Escherichia coli and Salmonella typhimurium HtrAs. An isogenic Y. pestis **htrA mutant** was constructed using a reverse genetics approach. In contrast to the wild-type strain, the mutant failed to grow at an elevated temp. of 39.degree.C, but showed only a small increase in sensitivity to oxidative stress and was only partially attenuated in the animal model. However, the mutant exhibited a different protein expression profile to that of the wild-type strain when grown at 28.degree.C to simulate growth in the flea.

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 5 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:189522 CAPLUS
DOCUMENT NUMBER: 133:16055

TITLE: Kinetics of the mucosal antibody secreting cell response and evidence of specific lymphocyte migration to the lung after oral **immunisation** with attenuated S. enterica var. typhimurium

AUTHOR(S): Allen, J. S.; Dougan, G.; Strugnell, R. A.
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Melbourne, Parkville, Australia

SOURCE: FEMS Immunol. Med. Microbiol. (2000), 27(4), 275-281
CODEN: FIMIEV; ISSN: 0928-8244
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The kinetic of mucosal secretory responses elicited by the **vaccine** vector Salmonella enterica var. typhimurium (S. typhimurium) was examd. by enzyme linked immunospot (ELISPOT) and compared with serum responses. Mice **immunized** orally with BRD509, the aroA, **aroD mutant** of virulent S. typhimurium SL1344 expressing the C Fragment of tetanus toxin (TT), simultaneously developed an IgA antibody secreting cells (ASC) response in the gastrointestinal lamina propria, the spleen and the lung, against both S. typhimurium lipopolysaccharide (LPS) and TT. The magnitude of the ASC response was greatest in the gut, was boosted by a secondary **immunization** at day 25, and the kinetic of the response did not correlate with the appearance of serum antibodies. This study suggests that S. typhimurium can engage the common mucosal immune system to effect mucosal secretory responses at distal sites, however, the magnitude of the responses is both greatest in the gut and antigen-specific. The ASC origin of the serum antibodies specific for S. typhimurium and antigens expressed by the **bacterium** is yet to be elucidated.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 6 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:626318 CAPLUS
DOCUMENT NUMBER: 131:253344
TITLE: **Bacteria** attenuated by a non-reverting mutation in each of the aroC, ompF and ompC genes, useful as **vaccines**

INVENTOR(S): Chatfield, Steven Neville
PATENT ASSIGNEE(S): Peptide Therapeutics Limited, UK

SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9949026	A1	19990930	WO 1999-GB935	19990325

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG,

SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW,
AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE,
DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9930458	A1	19991018	AU 1999-30458		19990325
EP 1066376	A1	20010110	EP 1999-911949		19990325
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
NO 2000004781	A	20001108	NO 2000-4781		20000925
PRIORITY APPLN. INFO.:			GB 1998-6449	A	19980325
			WO 1999-GB935	W	19990325

AB The invention provides a **bacterium** attenuated by a non-reverting mutation in each of the aroC gene, the ompF gene and the ompC gene. The **bacterium** is useful as a **vaccine**. The **bacterium** may, for example, be an attenuated strain of E. coli useful in **vaccination** against diarrhea. Thus, the design of deletions and construction of plasmids is described for removal of the entire open reading frame of target aroC, ompC, and ompF genes from the E1392/75/2A strain of enterotoxigenic E. coli. The attenuated **vaccine** strain (.DELTA.aroc/.DELTA.ompc/.DELTA.ompF) is well tolerated in healthy adult volunteers and colonizes the intestine in a manner consistent with its utility as an oral **vaccine** to protect against travelers diarrhea. It has also been demonstrated to elicit a specific mucosal immune response.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 7 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:388086 CAPLUS
DOCUMENT NUMBER: 131:43576
TITLE: **Vaccines** containing attenuated **bacteria**
INVENTOR(S): Chatfield, Steven Neville; Sydenham, Mark; Dougan, Gordon
PATENT ASSIGNEE(S): Medeva Europe Limited, UK
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9929342	A1	19990617	WO 1998-GB3680	19981210
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9914960	A1	19990628	AU 1999-14960	19981210

AU 739191 B2 20011004
EP 1037664 A1 20000927 EP 1998-959023 19981210
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
JP 2001525375 T2 20011211 JP 2000-524011 19981210
PRIORITY APPLN. INFO.: GB 1997-26233 A 19971211
WO 1998-GB3680 W 19981210

AB The invention relates to a **vaccine** comprising a **bacterium** attenuated by a non-reverting **mutation** in a gene, e.g. **surA** gene and gene for parvulin (peptidyl-prolyl cis-trans isomerase), encoding a protein which promotes folding of extracytoplasmic proteins. Such mutations were initially identified as being useful in **vaccines** from a bank of randomly inserted, transposon mutants in which attenuation was detd. as a redn. in virulence of the organism in the mouse model of infection. Site directed mutation of the gene results in a strain which shows at least 4 logs of attenuation when delivered both orally and i.v. Animals **vaccinated** with such a strain are protected against subsequent challenge with the parent wild type strain. Finally, heterologous antigens such as the non-toxic and protective, binding domain from tetanus toxin, fragment C, can be delivered via the mucosal immune system using such strains of **bacteria**. This results in the induction of a fully protective immune response to subsequent challenge with native tetanus toxin.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 8 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:296915 CAPLUS
DOCUMENT NUMBER: 129:53187
TITLE: Genetic control of immune response to recombinant antigens carried by an attenuated Salmonella typhimurium **vaccine** strain: Nramp1 influences T-helper subset responses and protection against leishmanial challenge
AUTHOR(S): Soo, Shiu-Shing; Villarreal-Ramos, Bernardo; Khan, C. M. Anjam; Hormaeche, Carlos E.; Blackwell, Jenefer M.
CORPORATE SOURCE: Department of Pathology, University of Cambridge, Cambridge, CB2 1QP, UK
SOURCE: Infect. Immun. (1998), 66(5), 1910-1917
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Attenuated strains of Salmonella typhimurium have been widely used as vehicles for delivery and expression of **vaccine** antigens in murine models of infectious disease. In mice, early **bacterial** replication following infection with S. typhimurium is controlled by the gene (Nramp1, formerly Ity/Lsh/Bcg) encoding the natural-resistance-assocd. macrophage protein (Nramp1). Nramp1 regulates macrophage activation and has multiple pleiotropic effects, including regulation of tumor necrosis factor alpha, interleukin 1.beta. (IL-1.beta.), and major histocompatibility complex class II mols., all of which influence antigen processing and presentation. Nramp1 also has a direct effect on antigen

processing, possibly by regulating the activity of proteases in the late endosomal compartment. Hence, there are multiple ways (regulation of **bacterial** load or recombinant antigen dose, class II mol. expression, costimulatory or adjuvant activity, and antigen processing) that Nramp1 might influence responses to recombinant salmonella **vaccines**. To test the hypothesis that Nramp1 influences responses to **vaccination**, congenic mouse strains have been used to analyze immune responses to recombinant antigens (tetanus toxoid antigen and leishmanial gp63) carried by live attenuated S. typhimurium aroA **aroD** **mutants**. Results show that congenic mice carrying the wild-type (S. typhimurium resistance) Nramp1 allele mount a predominantly T-helper-1 (IL-2 and gamma interferon) response to **vaccination** and show enhanced resoln. of lesions following challenge infection with Leishmania major. In contrast, mice carrying mutant (S. typhimurium susceptibility) Nramp1 mount a T-helper-2 (IgE and IL-4) response and show exacerbated lesion growth upon challenge.

L16 ANSWER 9 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:130763 CAPLUS
DOCUMENT NUMBER: 128:281571
TITLE: The **Haemophilus** influenzae HtrA protein is a protective antigen
AUTHOR(S): Loosmore, Sheena M.; Yang, Yan-Ping; Oomen, Ray; Shortreed, Jean M.; Coleman, Debbie C.; Klein, Michel H.
CORPORATE SOURCE: Pasteur Merieux Connaught Canada Research, North York, ON, M2R 3T4, Can.
SOURCE: Infect. Immun. (1998), 66(3), 899-906
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The htrA gene from two strains of nontypeable **Haemophilus** influenzae has been cloned and sequenced, and the encoded approx. 46-kDa HtrA proteins were highly conserved. H. influenzae HtrA has approx. 55% identity with the Escherichia coli and Salmonella typhimurium HtrA stress response proteins, and expression of the H. influenzae htrA gene was inducible by high temp. Recombinant HtrA (rHtrA) was expressed from E. coli, and the purified protein was found to have serine protease activity. RHtrA was very immunogenic and partially protective in both the passive infant rat model of bacteremia and the active chinchilla model of otitis media. Immunoblot anal. indicated that HtrA is antigenically conserved in encapsulated and nontypeable H. influenzae species. Site-directed **mutagenesis** was performed on the **htrA** gene to ablate the endogenous serine protease activity of wild-type **HtrA**, and it was found that eight of nine recombinant **mutant** proteins had no measurable residual proteolytic activity. Two mutant proteins were tested in the animal protection models, and one, H91A, was partially protective in both models. H91A HtrA may be a good candidate antigen for a **vaccine** against invasive H. influenzae type b disease and otitis media and is currently in phase I clin. trials.

L16 ANSWER 10 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:67764 CAPLUS

DOCUMENT NUMBER: 128:177998
TITLE: Intracellular multiplication and virulence of Shigella flexneri auxotrophic mutants
AUTHOR(S): Cersini, Antonella; Salvia, Anna Maria; Bernardini, Maria Lina
CORPORATE SOURCE: Dipartimento di Biologia Cellulare e dello Sviluppo, Fondazione Istituto Pasteur-Cenci Bolognetti, Universita di Roma "La Sapienza", Rome, 00185, Italy
SOURCE: Infect. Immun. (1998), 66(2), 549-557
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors have constructed and analyzed a group of Shigella flexneri 5 auxotrophic mutants. The wild-type strain M90T was mutagenized in genes encoding enzymes involved in the synthesis of (i) arom. amino acids, (ii) nucleotides, and (iii) diaminopimelic acid. In this way, strains with single (aroB, aroC, **aroD**, purE, thyA, and dapB) and double (purE aroB, purE aroC, purE **aroD**, purE thyA) **mutations** were obtained. Although the Aro mutants had the same nutritional requirements when grown in lab. media, they showed different degrees of virulence in vitro and in vivo. The aroB **mutant** was not significantly attenuated, whereas both the aroC and **aroD** strains were severely attenuated. P-Aminobenzoic acid (PABA) appeared to be the main requirement for the Aro mutants' growth in tissue culture. Concerning nucleotides, thymine reduced the pathogenicity, whereas adenine did not. However, when combined with another virulence-affecting mutation, adenine auxotrophy appeared to potentiate that mutation's effects. Consequently, the assocn. of either the purE and aroC or the purE and **aroD** **mutations** had a great effect on virulence as measured by the Sereny test, whereas the purE aroB double **mutation** appeared to have only a small effect. All mutants except the dapB strain seemed to move within a Caco-2 cell monolayer after 3 h of infection. Nevertheless, the auxotrophs showing a high intracellular generation time were neg. in the plaque assay. Knowledge of each mutation's role in attenuating Shigella strains will provide useful tools in designing **vaccine** candidates.

L16 ANSWER 11 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:746925 CAPLUS
DOCUMENT NUMBER: 128:58087
TITLE: Cloning and characterization of the aroA and aroD genes of Shigella dysenteriae type 1
AUTHOR(S): Walker, John C.; Verma, Naresh K.
CORPORATE SOURCE: Division of Biochemistry and Molecular Biology, Faculty of Science, School of Life Sciences, Australian National University, Canberra, 0200, Australia
SOURCE: Microbiol. Immunol. (1997), 41(10), 809-813
CODEN: MIIMDV; ISSN: 0385-5600
PUBLISHER: Center for Academic Publications Japan
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The aroA and aroD genes from Shigella dysenteriae type 1, encoding 5-enolpyruvylshikimate 3-phosphate synthase and 3-dehydroquinase,

resp., were cloned by polymerase chain reaction (PCR). Their nucleotide sequences were detd. and predicted to code for 46 kDa and 27.5 kDa proteins, resp. Protein expressed from these genes using the minicell system, corresponded to the size of the predicted protein products. The cloned genes were shown to be functional by complementation of Escherichia coli aroA- and **aroD-mutants**. The predicted amino acid sequences of the cloned aroA (427 amino acids) and aroD (252 amino acids) genes of S. dysenteriae type 1 were found to be highly homologous to the corresponding genes in other **bacterial** species, indicating the high conservation of these housekeeping genes. The use of the cloned aroA and aroD genes in the development of a **vaccine** strain against S. dysenteriae is discussed.

L16 ANSWER 12 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:738088 CAPLUS
DOCUMENT NUMBER: 128:72171
TITLE: The HtrA family of serine proteases
AUTHOR(S): Pallen, Mark J.; Wren, Brendan W.
CORPORATE SOURCE: Microbial Pathogenicity Research Group, Department of Medical Microbiology, St Bartholomew's and the Royal London School of Medicine and Dentistry, London, EC1A 7BE, UK
SOURCE: Mol. Microbiol. (1997), 26(2), 209-221
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with .apprx.70 refs. HtrA, also known as DegP and probably identical to the Do protease, is a heat shock-induced serine protease that is active in the periplasm of Escherichia coli. Homologues of HtrA have been described in a wide range of **bacteria** and in eukaryotes. Its chief role is to degrade misfolded proteins in the periplasm. Substrate recognition probably involves the recently described PDZ domains in the C-terminal half of HtrA and, we suspect, has much in common with the substrate recognition system of the tail-specific protease, Prc (which also possesses a PDZ domain). The expression of htrA is regulated by a complex set of signal transduction pathways, which includes an alternative sigma factor, RpoE, an anti-sigma factor, RseA, a two-component regulatory system, CpxRA, and two phosphoprotein phosphatases, PrpA and PrpB. **Mutations** in the **htrA** genes of Salmonella, **Brucella** and **Yersinia** cause decreased survival in mice and/or macrophages, and **htrA mutants** can act as **vaccines**, as cloning hosts and as carriers of heterologous antigens.

L16 ANSWER 13 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:358711 CAPLUS
DOCUMENT NUMBER: 127:120395
TITLE: Attenuated Salmonella typhi and Shigella as live oral **vaccines** and as live vectors
AUTHOR(S): Levine, M. M.; Galen, J.; Barry, E.; Noriega, F.; Tacket, C.; Sztein, M.; Chatfield, S.; Dougan, G.; Losonsky, G.; Kotloff, K.
CORPORATE SOURCE: School Medicine, Univ. Maryland, Baltimore, MD, 21201, USA

SOURCE: Behring Inst. Mitt. (1997), 98(New Approaches to Bacterial Vaccine Development), 120-123
CODEN: BHIMA2; ISSN: 0301-0457
PUBLISHER: Medizinische Verlagsgesellschaft mbH
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review is given with 26 refs. including the authors own works on new generations of attenuated Salmonella typhi and Shigella strains with precise, defined **mutations** for use as live oral **vaccines** and on the live vectors CVD 908 and CVD 908-**htrA**.

L16 ANSWER 14 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:229145 CAPLUS
DOCUMENT NUMBER: 126:304818
TITLE: Salmonella typhimurium aroA, **htrA**, and **aroD htrA mutants** cause progressive infections in athymic (nu/nu) BALB/c mice
AUTHOR(S): Sinha, Katharine; Mastroeni, Pietro; Harrison, Julia; Demarco de Hormaeche, Raquel; Hormaeche, Carlos E.
CORPORATE SOURCE: Department of Microbiology, The Medical School, University of Newcastle, Newcastle upon Tyne, NE2 4HH, UK
SOURCE: Infect. Immun. (1997), 65(4), 1566-1569
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Athymic (nu/nu) BALB/c mice and their euthymic (nu/+) littermates were inoculated i.v. with live attenuated **vaccine** strains of Salmonella typhimurium. All strains caused progressive infections in the athymic mice but not in their euthymic littermates. Athymic mice given strain SL3261, an aroA deriv. of SL1344, in doses between log 4.7 and 5.7 CFU were all severely ill and were killed by weeks 4 to 5. Athymic mice given log 4.7 CFU of a deriv. of S. typhimurium C5 carrying a **mutation** in **htrA**, encoding a stress protein, were ill and were killed by week 7 in one expt. but survived to week 13 in another. Athymic mice given log 4.6 CFU of a C5 **aroD htrA** double **mutant** were ill and were killed at week 7. Athymic mice given SL3261 had high **bacterial** counts in the reticuloendothelial system at 4 wk. Athymic mice given SL3261 or C5 htrA made IgG3 (and to a lesser extent IgM) antibody to lipopolysaccharide (LPS), whereas euthymic mice made IgM, IgG1, IgG2a, IgG2b, and IgG3 anti-LPS antibodies. The results indicate that both aroA and htrA strains will produce slow, progressively lethal infections in athymic mice, that the htrA strain is more attenuated than the aroA strain as measured by time to death in this model, and that IgG3 anti-LPS antibody alone cannot suppress the progress of infections by very attenuated strains in athymic mice.

L16 ANSWER 15 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:647040 CAPLUS
DOCUMENT NUMBER: 125:295804
TITLE: Cloning, sequencing, expression, purification and preliminary characterization of a type II

dehydroquinase from Helicobacter pylori
AUTHOR(S): Bottomley, Joanna R.; Clayton, Christopher L.; Chalk, Peter A.; Kleanthous, Colin
CORPORATE SOURCE: Sch. Biological Sci., Univ. East Anglia, Norwich, NR4 7TJ, UK
SOURCE: Biochem. J. (1996), 319(2), 559-565
CODEN: BIJOAK; ISSN: 0264-6021
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A heat-stable dehydroquinase was purified to near homogeneity from a plate-grown suspension of the Gram-neg. stomach pathogen Helicobacter pylori, and shown from both its subunit and native mol. masses to be a member of the type II family of dehydroquinases. This was confirmed by N-terminal amino acid sequence data. The gene encoding this activity was isolated following initial identification, by random sequencing of the H. pylori genome, of a 96 bp fragment, the translated sequence of which showed strong identity to a C-terminal region of other type II enzymes. Southern blot anal. of a cosmid library identified several potential clones, one of which complemented an Escherichia coli **aroD** point **mutant** strain deficient in host dehydroquinase. The gene encoding the H. pylori type II dehydroquinase (designated aroQ) was sequenced. The translated sequence was identical to the N-terminal sequence obtained directly from the purified protein, and showed strong identity to other members of the type II family of dehydroquinases. The enzyme was readily expressed in E. coli from a plasmid construct from which several milligrams of protein could be isolated, and the mol. mass of the protein was confirmed by electrospray MS. The aroQ gene in H. pylori may function in the central biosynthetic shikimate pathway of this **bacterium**, thus opening the way for the construction of attenuated strains as potential **vaccines** as well as offering a new target for selective enzyme inhibition.

L16 ANSWER 16 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:140929 CAPLUS
DOCUMENT NUMBER: 118:140929
TITLE: Cloning of dapD, aroD and asd of Leptospira interrogans serovar icterohaemorrhagiae, and nucleotide sequence of the asd gene
AUTHOR(S): Baril, Celine; Richaud, Catherine; Fournie, Edith; Baranton, Guy; Saint Girons, Isabelle
CORPORATE SOURCE: Unite Bacteriol. Mol. Med., Inst. Pasteur, Paris, 75724, Fr.
SOURCE: J. Gen. Microbiol. (1992), 138(1), 47-53
CODEN: JGMIAN; ISSN: 0022-1287
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Metabolites such as diaminopimelate and some arom. derivs., not synthesized in mammalian cells, are essential for growth of **bacteria**. As a first step towards the design of a new human live **vaccine** that uses attenuated strains of L. interrogans, the asd, **aroD**, and dapD genes, encoding aspartate .beta.-semialdehyde dehydrogenase, 3-dehydroquinase, and tetrahydrodipicolinate N-succinyltransferase, resp., were cloned by complementation of Escherichia coli **mutants**. The complete nucleotide sequence of the asd gene was detd. and found to contain an open reading frame capable of encoding a protein of 349 amino

acids with a calcd. Mr of 38,007. Comparison of this deduced L. interrogans aspartate .beta.-semialdehyde dehydrogenase amino acid sequence with those of the same enzyme from Saccharomyces cerevisiae and Corynebacterium glutamicum revealed 46% and 36% identity, resp. By contrast, the identity between the L. interrogans enzyme and the Streptococcus mutans or E. coli enzymes was less than 31%. Highly conserved sequences within aspartate semialdehyde dehydrogenase from the 5 organisms were obsd. at the amino and carboxyl termini, and around the cysteine of the active site.

L16 ANSWER 17 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:109672 CAPLUS
DOCUMENT NUMBER: 118:109672
TITLE: Attenuated **bacteria** expressing antigenic protein genes and their use as **vaccines**
INVENTOR(S): Charles, Ian George; Chatfield, Steven Neville; Fairweather, Neil Fraser
PATENT ASSIGNEE(S): Wellcome Foundation Ltd., UK
SOURCE: PCT Int. Appl., 23 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9215689	A1	19920917	WO 1992-GB387	19920305
W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US				
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
AU 9213508	A1	19921006	AU 1992-13508	19920305
AU 664360	B2	19951116		
EP 574466	A1	19931222	EP 1992-905914	19920305
EP 574466	B1	19990519		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL				
JP 06505158	T2	19940616	JP 1992-505563	19920305
HU 66833	A2	19950130	HU 1993-2492	19920305
HU 219535	B	20010528		
PL 170938	B1	19970228	PL 1992-296702	19920305
PL 171476	B1	19970530	PL 1992-312415	19920305
RU 2126447	C1	19990220	RU 1993-57957	19920305
CZ 285118	B6	19990512	CZ 1993-1005	19920305
AT 180280	E	19990615	AT 1992-905914	19920305
ES 2131069	T3	19990716	ES 1992-905914	19920305
NO 9302423	A	19930702	NO 1993-2423	19930702
US 5547664	A	19960820	US 1994-354776	19941212
US 5683700	A	19971104	US 1995-469507	19950606

PRIORITY APPLN. INFO.:
GB 1991-4596 A 19910305
GB 1991-21208 A 19911004
WO 1992-GB387 A 19920305
US 1993-81361 B1 19930630
US 1994-246773 B1 19940520
US 1994-354776 A3 19941212

AB Attenuated **bacteria** contg. an antigenic protein gene fused to a promoter whose activity is induced by anaerobic conditions are

described. These transformants can be used as **vaccines**. Salmonella typhimurium (aroA-aroD-) were transformed with a plasmid contg. the gene for tetanus toxin fragment C fused to the nirB promoter of Escherichia coli. These **bacteria** were effective single-dose oral **vaccines** against tetanus toxin challenge in mice.

L16 ANSWER 18 OF 21 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:5215 CAPLUS
DOCUMENT NUMBER: 118:5215
TITLE: Genotypic and phenotypic characterization of an aroD deletion-attenuated Escherichia coli K12-Shigella flexneri hybrid **vaccine** expressing S. flexneri 2a somatic antigen
AUTHOR(S): Newland, John W.; Hale, Thomas Larry; Formal, Samuel B.
CORPORATE SOURCE: Dep. Enteric Infect., Walter Reed Army Inst. Res., Washington, DC, 20307, USA
SOURCE: Vaccine (1992), 10(11), 766-76
CODEN: VACCDE; ISSN: 0264-410X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The construction and characterization of EcSf2a-2, an aroD-deleted E. coli-Shigella hybrid **vaccine** carrying chromosomal and plasmid genes from S. flexneri and expressing S. flexneri 2a somatic antigen in assocn. with E. coli K12 core are described. Expression of hybrid lipopolysaccharide and deletion of aroD resulted in the attenuation of phenotypic characteristics assocd. with pathogenicity. The addn. of an aroD deletion results in a requirement for an arom. precursor of para-aminobenzoic acid (PABA), an essential **bacterial** metabolite not present in mammalian tissues. The biosynthesis of hybrid somatic antigen prevents expression of a Sereny-pos. reaction by invasive **bacteria** capable of expressing a plaque-pos. phenotype. A functional kcpA gene is required for expression of the plaque-pos. phenotype. The presence of an aroD deletion does not interfere with expression of an invasive phenotype; however, in **bacteria** contg. a functional kcpA gene, replication and spread by invading **bacteria** are limited, preventing development of the plaque-pos. phenotype.

L16 ANSWER 19 OF 21 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1992:241920 CAPLUS
DOCUMENT NUMBER: 116:241920
TITLE: Acid-tolerant Salmonella typhi, double aro mutants thereof, and use as an oral **vaccine** for typhoid fever
INVENTOR(S): Hone, David M.; Levine, Myron M.
PATENT ASSIGNEE(S): University of Maryland, Baltimore, USA
SOURCE: PCT Int. Appl., 104 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 9118092 A1 19911128 WO 1991-US3447 19910522
W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE
PRIORITY APPLN. INFO.: US 1990-527467 19900523
AB Acid-tolerant S. typhi are isolated. aro Double mutants of these strains may be used as oral **vaccines** for typhoid fever. Addnl., these acid-tolerant S. typhi can be used as carriers of antigen genes from other pathogens, and can therefore serve as an oral **vaccine** for these pathogens as well. A method for enriching for and isolating acid-tolerant S. typhi comprising culture in acidic, buffered medium at low O tension was developed. An S. typhi strain contg. deletions in the aroC and aroD genes was prepd. The LD50 for this strain in mice was 2.9 .times. 107 (relative to <150 for the wild-type parent). The mutations did not cause any change in the capsular, somatic, or flagellar antigens. Ingestion of this mutant by human volunteers resulted in appearance of antibody-secreting cells and significant antibody-dependent cell-mediated cytotoxic response.

L16 ANSWER 20 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:84145 CAPLUS
DOCUMENT NUMBER: 112:84145
TITLE: Live **vaccines** containing attenuated microorganisms having double mutations in genes in the aromatic biosynthetic pathway
INVENTOR(S): Dougan, Gordon; Chatfield, Steven Neville
PATENT ASSIGNEE(S): Wellcome Foundation Ltd., UK
SOURCE: Eur. Pat. Appl., 13 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 322237	A1	19890628	EP 1988-312203	19881222
EP 322237	B1	19940323		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
WO 8905856	A1	19890629	WO 1988-GB1143	19881222
W: AU, BR, DK, HU, JP, SU, US				
AU 8929193	A1	19890719	AU 1989-29193	19881222
AU 619519	B2	19920130		
BR 8807376	A	19900320	BR 1988-7376	19881222
ZA 8809605	A	19900829	ZA 1988-9605	19881222
JP 02502785	T2	19900906	JP 1989-501160	19881222
HU 55242	A2	19910528	HU 1989-702	19881222
HU 216449	B	19990628		
CA 1327331	A1	19940301	CA 1988-586868	19881222
AT 103331	E	19940415	AT 1988-312203	19881222
ES 2061700	T3	19941216	ES 1988-312203	19881222
IL 88766	A1	19950731	IL 1988-88766	19881222
KR 9710759	B1	19970630	KR 1988-17199	19881222
RU 2114172	C1	19980627	RU 1988-4742131	19881222
DK 8904126	A	19890822	DK 1989-4126	19890822
US 5811105	A	19980922	US 1995-449297	19950524
US 5770214	A	19980623	US 1995-484314	19950607

PRIORITY APPLN. INFO.: GB 1987-30037 A 19871223

EP 1988-312203	A	19881222
WO 1988-GB1143	A	19881222
US 1989-399539	B1	19890822
US 1991-642138	B1	19910115
US 1992-857092	B1	19920320
US 1992-979460	B1	19921120
US 1993-135436	B3	19931013

AB An attenuated microorganism harboring 2 mutated genes, each of which is located in the organism's arom. biosynthetic pathway is useful as a **vaccine**. The attenuated microorganism can be genetically engineered so as to express antigens from other pathogens, thus making a range of multivalent **vaccines**. Salmonella typhimurium aroA aroC double mutant was prepd. by transposon mutagenesis. Balb/c mice treated by oral administration of 109-1010 of the mutant resisted oral challenge by the parental virulent strain (SL 1344) of S. typhimurium 28 and 70 days post **immunization**. Oral tablets contained freeze-dried S. typhi double mutant 70.0, Aerosil-200 0.5, Dipac 235.0, crosslinked Povidone 7.0, microcryst. cellulose, 35.0, and Mg stearate 2.5 mg coated with Opadry Enteric OY-P-7156 35.0 mg.

L16 ANSWER 21 OF 21 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:52059 CAPLUS
DOCUMENT NUMBER: 112:52059
TITLE: Transposon-generated Tn10 insertion mutations at the aro genes of Escherichia coli K-12
AUTHOR(S): Cobos, Antonio; Fernandez, Maria F.; Hernandez, Pablo E.; Sanz, Bernabe
CORPORATE SOURCE: Fac. Vet. Med., Univ. Complutense, Madrid, 28040, Spain
SOURCE: Curr. Microbiol. (1990), 20(1), 13-18
CODEN: CUMIDD; ISSN: 0343-8651
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A set of E. coli K-12 derivs. was prepd. with transposon-generated Tn10 insertion mutations at the aro genes for the arom. biosynthetic pathway. Bacteriophage .lambda.NK561 (Tn10) was used for transposon mutagenesis of E. coli strain BW545. Tetracycline (Tc)-resistant derivs. were screened by their Aro- phenotype by growth on a minimal medium with adequate requirements. Six aro **mutant** types were mapped: two strains were aroA, two **aroD**, one aroB or **aroE**, and one aroC. A selective medium and a D-cycloserine enrichment in the presence of tetracycline were used to select for Aro-, Tc-sensitive derivs. The reversion index to arom.-independent colonies of some derivs. was <2 .times. 10-11/**bacterium** /generation. PI transduction expts. transferred an aroA::Tn10 insertion from E. coli BW545 to an enterotoxigenic E. coli strain from porcine origin. Derivs. of this strain being aro, Tc-sensitive and not reverting to aro+ at a detectable frequency, and many others transduced at will, may prove their usefulness as live **vaccines**.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 12:46:31 ON 06 FEB 2002)
L17 122 S L16
L18 59 DUP REM L17 (63 DUPLICATES REMOVED)

L18 ANSWER 1 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD

ACCESSION NUMBER: 2002-049352 [06] WPIDS
DOC. NO. CPI: C2002-013898
TITLE: Microorganism useful as a **vaccine** for **immunizing** vertebrates, comprises a regulated antigen delivery system with a runaway vector and genes encoding a repressor whose synthesis is under control of an activatible control sequence.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): CURTISS, R; TINGE, S A
PATENT ASSIGNEE(S): (MEGA-N) MEGAN HEALTH INC; (UNIW) UNIV WASHINGTON
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001083785	A2	20011108	(200206)*	EN	95

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001083785	A2	WO 2001-US13915	20010430

PRIORITY APPLN. INFO: US 2000-560539 20000428
AN 2002-049352 [06] WPIDS
AB WO 200183785 A UPAB: 20020128
NOVELTY - A microorganism (I) comprising a regulated antigen delivery system (RADS), comprising:
(a) a vector (II) having:
(i) a site (SI) for insertion of a desired gene; and
(ii) a first origin of replication (ori) and a second ori conferring vector replication using DNA polymerase III and I, respectively; and
(b) a gene (III) encoding a first repressor (FR) operably linked to a first activatible control sequence, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a runaway vector (IV) comprising (II);
(2) producing a desired gene product comprising:
(a) engineering a gene encoding the desired product into the vector of (I), where the microorganism comprises control sequences that repress expression of the second ori under an environmental condition, but in which the expression of the second ori is derepressed under a second environmental condition;
(b) culturing (I) under the first environmental condition; and
(c) culturing the microorganism with the vector of (a) under the second environmental condition;
(3) a **vaccine** (V) for **immunization** of a vertebrate, where (V) comprises (I) in a carrier;

(4) inducing immunoprotection in a vertebrate comprising administering (V); and

(5) delivering a desired gene product to a vertebrate comprising administering (I).

ACTIVITY - Antibacterial; immunostimulant.

MECHANISM OF ACTION - **Vaccine** (claimed). The immunogenic properties of the RAV SeM **vaccine** strains were initially evaluated in BALB/c mice given about 107 colony forming units (CFU) of each strain intranasally on day 0 and day 28 without anesthesia. Only low levels of **vaccine** strains were recovered from the Lungs and Peyer's patches of the **immunized** mice 72 hours following **immunization** and similarly were rarely detected in feces of **immunized** mice following day 3. The serological immunoglobulin (Ig)G SeM specific antibody response detected indicated that all strains induced strong antibody immune response to the SeM antigen.

USE - (I) is useful for producing a desired gene product, preferably an antigen which is Ery65 or SeM. (I) is useful for delivering a desired gene product in a vertebrate. A **vaccine** (V) comprising (I) is useful for inducing immunoprotection in a vertebrate against antigens such as Ery65 which causes disease erysipelas and in later life can cause arthritis in swine and turkeys, and SeM which causes strangles in racehorses and other equines (all claimed).

ADVANTAGE - As a **vaccine**, the RADS is capable of causing an effective exposure of the **immunized** vertebrate's lymphoid tissues to a large dose of vector-encoded foreign gene product production in response to the withdrawal of the stimulus. The RADS microorganism can be grown in vitro under low copy number control, then switched to runaway conditions after vertebrate inoculation to cause an increase in antigen production in vivo. Under derepressed runaway conditions, the RADS microorganisms is highly impaired due to extremely high plasmid replication activity coupled with extremely high foreign gene product production. Because of its impaired state, the derepressed RADS microorganisms cannot generally survive for extended periods.

Dwg.0/23

L18 ANSWER 2 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 1

ACCESSION NUMBER:	2001:397600 BIOSIS
DOCUMENT NUMBER:	PREV200100397600
TITLE:	Construction, genotypic and phenotypic characterization, and immunogenicity of attenuated DELTAguaBA Salmonella enterica serovar Typhi strain CVD 915.
AUTHOR(S):	Wang, Jin Yuang; Pasetti, Marcela F.; Noriega, Fernando R.; Anderson, Richard J.; Wasserman, Steven S.; Galen, James E.; Sztein, Marcelo B.; Levine, Myron M. (1)
CORPORATE SOURCE:	(1) Center for Vaccine Development, University of Maryland School of Medicine, 685 W. Baltimore St., Baltimore, MD, 21201: mlevine@medicine.umaryland.edu USA
SOURCE:	Infection and Immunity, (August, 2001) Vol. 69, No. 8, pp. 4734-4741. print. ISSN: 0019-9567.
DOCUMENT TYPE:	Article

LANGUAGE: English
SUMMARY LANGUAGE: English
AB A promising live attenuated typhoid **vaccine** candidate strain for mucosal **immunization** was developed by introducing a deletion in the guaBA locus of pathogenic Salmonella enterica serovar Typhi strain Ty2. The resultant DELTAguaBA **mutant**, serovar Typhi CVD 915, has a gene encoding resistance to arsenite replacing the deleted sequence within guaBA, thereby providing a marker to readily identify the **vaccine** strain. CVD 915 was compared in in vitro and in vivo assays with wild-type strain Ty2, licensed live oral typhoid **vaccine** strain Ty21a, or attenuated serovar Typhi **vaccine** strain CVD 908-**htrA** (harboring **mutations** in aroC, **aroD**, and **htrA**). CVD 915 was less invasive than CVD 908-**htrA** in tissue culture and was more crippled in its ability to proliferate after invasion. In mice inoculated intraperitoneally with serovar Typhi and hog gastric mucin (to estimate the relative degree of attenuation), the 50% lethal dose of CVD 915 (7.7 X 107 CFU) was significantly higher than that of wild-type Ty2 (1.4 X 102 CFU) and was only slightly lower than that of Ty21a (1.9 X 108 CFU). Strong serum O and H antibody responses were recorded in mice inoculated intranasally with CVD 915, which were higher than those elicited by Ty21a and similar to those stimulated by CVD 908-**htrA**. CVD 915 also elicited potent proliferative responses in splenocytes from **immunized** mice stimulated with serovar Typhi antigens. Used as a live vector, CVD 915(pTETlpp) elicited high titers of serum immunoglobulin G antifragment C. These encouraging preclinical data pave the way for phase 1 clinical trials with CVD 915.

L18 ANSWER 3 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-465747 [40] WPIDS
DOC. NO. CPI: C2000-140265
TITLE: **Bacteria** deficient in activity of protease HtrA, useful for production of proteins, e.g. **vaccinating** antigens, that are exported from the cell, provide increased yield.
DERWENT CLASS: B04 D16
INVENTOR(S): BOLOTINE, A; GRUSS, A; POQUET, I; SOROKINE, A
PATENT ASSIGNEE(S): (INRG) INRA INST NAT RECH AGRONOMIQUE; (INRG) INST NAT RECH AGRONOMIQUE
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000039309	A1	20000706	(200040)*	FR	41
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
FR 2787810	A1	20000630	(200040)		
AU 2000017863	A	20000731	(200050)		
EP 1141337	A1	20011010	(200167)	FR	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000039309	A1	WO 1999-FR3270	19991223
FR 2787810	A1	FR 1998-16462	19981224
AU 2000017863	A	AU 2000-17863	19991223
EP 1141337	A1	EP 1999-961158	19991223
		WO 1999-FR3270	19991223

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000017863	A	Based on	WO 200039309
EP 1141337	A1	Based on	WO 200039309

PRIORITY APPLN. INFO: FR 1998-16462 19981224
AN 2000-465747 [40] WPIDS
AB WO 200039309 A UPAB: 20000823
NOVELTY - Production of a protein (I) comprising culturing a **bacterial** strain (A) that expresses (I) and is prepared from a Gram-positive species with a genome no larger than 3.2 Mb by **mutational** inactivation of the **HtrA** surface protease, and recovering (I) exported from the cells, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for (A) comprising an expression cassette for (I), but excluding Lactobacillus helveticus that contains a single cassette consisting of the gusA reporter gene inserted into the htrA gene and under control of the promoter of this gene.

USE - (A) are used for preparation of fermented products, therapeutic proteins (especially **vaccines**) or dietary proteins (claimed) (e.g. for the production of cheeses or enzymes for facilitating digestion).

ADVANTAGE - Inactivation of HtrA almost completely eliminates degradation of exported proteins, so increases yields of (I) and prevents contamination by proteolytic degradation products (facilitating purification at reduced cost). Increased yields of (I) also improve maturation and organoleptic qualities of fermented foods. Inactivation of HtrA also affects survival of **bacteria** under stress, so may contribute to attenuation of **vaccinating** strains.
Dwg.0/7

L18 ANSWER 4 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-302849 [26] WPIDS
DOC. NO. CPI: C2000-091734
TITLE: New live attenuated Salmonella **vaccines** used for protecting poultry against infection by avian pathogenic gram-negative **bacteria** comprise an rfb/rfc gene cluster of the **bacteria** stably integrated in Salmonella chromosome.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): ROLAND, K L
PATENT ASSIGNEE(S): (MEGA-N) MEGAN HEALTH INC
COUNTRY COUNT: 87

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000004919	A2	20000203	(200026)*	EN	48
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9949914	A	20000214	(200029)		
EP 1100536	A2	20010523	(200130)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
ZA 2001000976	A	20011031	(200173)		70
CN 1315871	A	20011003	(200205)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000004919	A2	WO 1999-US15842	19990713
AU 9949914	A	AU 1999-49914	19990713
EP 1100536	A2	EP 1999-933977	19990713
		WO 1999-US15842	19990713
ZA 2001000976	A	ZA 2001-976	20010205
CN 1315871	A	CN 1999-810045	19990713

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9949914	A	Based on	WO 200004919
EP 1100536	A2	Based on	WO 200004919

PRIORITY APPLN. INFO: US 1998-122441 19980724

AN 2000-302849 [26] WPIDS

AB WO 200004919 A UPAB: 20000531

NOVELTY - A **vaccine** (I) for **immunization** of birds against an avian pathogenic gram-negative (APGN) microbe (II), is new.

DETAILED DESCRIPTION - A **vaccine** (I) for **immunization** of birds against an avian pathogenic gram-negative (APGN) microbe (II), is new and comprises live cells of a recombinant Salmonella strain (III) expressing an O-antigen of (II), and having:

(1) a rfb/rfc gene cluster of (II) stably integrated into the Salmonella chromosome; and

(2) a mutation in the rfb gene cluster or rfc gene of (III) which inactivates expression of the O-antigen,

where (III) is an attenuated mutant of a virulent Salmonella strain.

INDEPENDENT CLAIMS are also included for the following:

(1) a method (IV) for **immunizing** a bird against an APGN microbe, comprising administering (I) to the bird;

(2) a **vaccine** (V) for **immunization** of birds against at least two APGN microbes, comprising a mixture of live

cells of first and second recombinant Salmonella strains, each strain having the features of (1) and (2) above;

(3) a **vaccine** (VI) for **immunization** of birds against at least two APGN microbes, comprising live cells of a recombinant Salmonella strain expressing an O-antigen of each of the APGN microbes, and having a rfb/rfc gene cluster of each of the APGN microbes stably integrated into the Salmonella chromosome, and having a mutation in the Salmonella rfb gene cluster or rfc gene which inactivates expression of the Salmonella O-antigen, wherein the recombinant Salmonella strain is an attenuated mutant of a virulent Salmonella strain; and

(4) a method (VII) of making a **vaccine** for **immunizing** a bird against an APGN microbe.

USE - The **vaccines** are used to **immunize** birds against pathogenic gram negative **bacteria**, especially avian pathogenic Escherichia coli (APEC), which cause diseases such as air sacculitis, cellulitis, colibacillosis, and peritonitis. Birds which may be **immunized** include geese, pheasants, and other domesticated birds, especially chickens and turkeys as well as non-domesticated birds such as parrots and parakeets. The recombinant Salmonella strain can also be used to deliver a desired gene product to the **vaccinated** bird. The avirulent microbes can be used as vectors for the synthesis of other proteins, including immunoregulatory molecules made by avian species that might stimulate or suppress various physiological functions such as growth rate, fat or protein content.

ADVANTAGE - As (I) is an oral **vaccine**, it costs less to produce and is easier to administer in the field than an injectable **vaccine**. The recombinant Salmonella strain protects against both the gram negative microbe and the parental Salmonella strain. Also, as Salmonella sp. persist in the gut, they provide a more vigorous immune response.

Dwg.0/5

L18 ANSWER 5 OF 59 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 2001021219 MEDLINE
DOCUMENT NUMBER: 20448972 PubMed ID: 10992518
TITLE: Comparison of abilities of Salmonella enterica serovar typhimurium aroA **aroD** and aroA **htrA mutants** to act as live vectors.
AUTHOR: Roberts M; Chatfield S; Pickard D; Li J; Bacon A
CORPORATE SOURCE: Department of Veterinary Pathology, Glasgow University Veterinary School, Glasgow G61 1QH, United Kingdom.. M.Roberts@vet.gla.ac.uk
SOURCE: INFECTION AND IMMUNITY, (2000 Oct) 68 (10) 6041-3. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001103

AB We compared the ability of Salmonella enterica serovar Typhimurium SL1344 aroA **aroD** (BRD509) and aroA **htrA** (BRD807) **mutants** to act as live vectors for delivery of fragment C of

tetanus toxin (FrgC). FrgC was expressed in these strains from either pTETnir15 or pTEThtrA1. BRD509FrgC(+) strains elicited approximately 2-log-higher serum anti-FrgC antibody titers than BRD807FrgC(+) strains. All mice **immunized** with BRD807pTEThtrA1, BRD509pTEThtrA1, and BRD509pTETnir15 (but not BRD807pTETnir15) were protected against tetanus.

L18 ANSWER 6 OF 59 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000278088 MEDLINE
DOCUMENT NUMBER: 20278088 PubMed ID: 10816454
TITLE: Mucosal and systemic immune responses to chimeric fimbriae expressed by Salmonella enterica serovar typhimurium **vaccine** strains.
AUTHOR: Chen H; Schifferli D M
CORPORATE SOURCE: Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania 19104, USA.
CONTRACT NUMBER: CA-16520 (NCI)
DK-19525 (NIDDK)
SOURCE: INFECTION AND IMMUNITY, (2000 Jun) 68 (6) 3129-39.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000706
Last Updated on STN: 20000706
Entered Medline: 20000623

AB Recombinant live oral **vaccines** expressing pathogen-derived antigens offer a unique set of attractive properties. Among these are the simplicity of administration, the capacity to induce mucosal and systemic immunity, and the advantage of permitting genetic manipulation for optimal antigen presentation. In this study, the benefit of having a heterologous antigen expressed on the surface of a live vector rather than intracellularly was evaluated. Accordingly, the immune response of mice **immunized** with a Salmonella enterica serovar Typhimurium **vaccine** strain expressing the Escherichia coli 987P fimbrial antigen on its surface (Fas(+)) was compared with the expression in the periplasmic compartment (Fas(-)). Orally **immunized** BALB/c mice showed that 987P fimbriated Salmonella serovar Typhimurium CS3263 (aroA asd) with pCS151 (fas(+) asd(+)) elicited a significantly higher level of 987P-specific systemic immunoglobulin G (IgG) and mucosal IgA than serovar Typhimurium CS3263 with pCS152 (fasD **mutant** , asd(+)) expressing 987P periplasmic antigen. Further studies were aimed at determining whether the 987P fimbriae expressed by serovar Typhimurium chi4550 (cya crp asd) could be used as carriers of foreign epitopes. For this, the **vaccine** strain was genetically engineered to express chimeric fimbriae carrying the transmissible gastroenteritis virus (TGEV) C (379-388) and A (521-531) epitopes of the spike protein inserted into the 987P major fimbrial subunit FasA. BALB/c mice administered orally serovar Typhimurium chi4550 expressing the chimeric fimbriae from the tet promoter in pCS154 (fas(+) asd(+)) produced systemic antibodies against both fimbria and the TGEV C epitope but not against the TGEV A epitope. To improve the immunogenicity of the chimeric fimbriae, the in vivo inducible **nirB** promoter was inserted into

pCS154, upstream of the fas genes, to create pCS155. In comparison with the previously used **vaccine**, BALB/c mice **immunized** orally with serovar Typhimurium chi4550/pCS155 demonstrated significantly higher levels of serum IgG and mucosal IgA against 987P fimbria. Moreover, mucosal IgA against the TGEV C epitope was only detected with serovar Typhimurium chi4550/pCS155. The induced antibodies also recognized the epitopes in the context of the full-length TGEV spike protein. Hence, immune responses to heterologous chimeric fimbriae on Salmonella **vaccine** vectors can be optimized by using promoters known to be activated in vivo.

L18 ANSWER 7 OF 59 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000412946 EMBASE
TITLE: Susceptibility of calves to challenge with Salmonella typhimurium 4/74 and derivatives harbouring **mutations** in **htrA** or purE.
AUTHOR: Villarreal-Ramos B.; Manser J.M.; Collins R.A.; Chance V.; Eckersall P.D.; Jones P.W.; Dougan G.
CORPORATE SOURCE: B. Villarreal-Ramos, Institute for Animal Health, Compton, Berkshire RG20 7NN, United Kingdom. Bernardo.Villarreal@bbsrc.ac.uk
SOURCE: Microbiology, (2000) 146/11 (2775-2783).
Refs: 37
ISSN: 1350-0872 CODEN: MROBEO
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Salmonella typhimurium 4/74 is highly virulent for cattle after oral challenge, causing severe diarrhoea, which is sometimes associated with systemic spread of the micro-organism. Although susceptible to oral challenge, groups of cattle were found to be relatively resistant to subcutaneous challenge with this strain. The virulence of S. typhimurium 4/74 harbouring **mutations** in **htrA** and purE was also assessed in cattle. Although S. typhimurium 4/74 **htrA** and purE are attenuated following oral challenge in mice, cattle were highly susceptible to oral challenge with these **mutants**. As with the parent S. typhimurium 4/74 strain, cattle exhibited greater susceptibility to oral compared to subcutaneous challenge with S. typhimurium **htrA** and purE **mutants**. Following subcutaneous challenge with sublethal levels of S. typhimurium 4/74, calves produced significant levels of antibodies to S. typhimurium soluble extract. No correlation was detected between interferon gamma levels in sera and susceptibility to infection by any route. The concentrations of the acute-phase-associated protein haptoglobin were increased in the sera of five of six cattle inoculated subcutaneously, although increases in concentration were smaller in cattle inoculated orally.

L18 ANSWER 8 OF 59 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2000087308 MEDLINE
DOCUMENT NUMBER: 20087308 PubMed ID: 10618549
TITLE: Cattle immune responses to tetanus toxoid elicited by

recombinant S. typhimurium **vaccines** or tetanus toxoid in alum or Freund's adjuvant.

AUTHOR: Villarreal-Ramos B; Manser J M; Collins R A; Dougan G; Howard C J
CORPORATE SOURCE: Institute for Animal Health, Compton, Newbury, UK.. bernardo.villareal@bbsrc.ac.uk

SOURCE: VACCINE, (2000 Feb 14) 18 (15) 1515-21.
Journal code: X60; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000330
Last Updated on STN: 20000330
Entered Medline: 20000321

AB Cattle were **immunised** orally, nasally or subcutaneously with either S. typhimurium 4/74 aroA(-) **aroD**(-) or S. typhimurium 4/74 **htrA**-based live **vaccines** expressing Fragment C (TetC) of tetanus toxin from plasmid pTetnir15. Oral inoculation with S. typhimurium 4/74 aroA(-) **aroD**(-)- (pTetnir15) elicited mucosal anti-TetC IgA but no measurable systemic humoral responses to TetC. Subcutaneous inoculation with the same strain elicited both mucosal IgA and systemic anti-TetC IgG1 responses. Nasal inoculation did not elicit any detectable anti-TetC responses. Oral delivery of S. typhimurium **htrA**(-) proved fatal in inoculated animals. None of the animals inoculated with either **mutant** S. typhimurium developed detectable T cell proliferative responses to the guest antigen. Cattle were also inoculated with tetanus toxoid adsorbed in alum or emulsified in Freund's complete adjuvant. Animals inoculated subcutaneously with Ttox emulsified in FCA developed systemic IgG1 and IgG2 antibody, while animals inoculated with Ttox adsorbed in alum developed systemic IgG1 but little IgG2 to Ttox. Both of these groups of animals developed measurable TetC-specific proliferative T cell responses that were associated with the production of IFNgamma.

L18 ANSWER 9 OF 59 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 2000143713 MEDLINE
DOCUMENT NUMBER: 20143713 PubMed ID: 10678914

TITLE: Salmonella enterica serovar typhimurium **surA mutants** are attenuated and effective live oral **vaccines**.

AUTHOR: Sydenham M; Douce G; Bowe F; Ahmed S; Chatfield S; Dougan G
CORPORATE SOURCE: Medeva Vaccine Development Group, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ, United Kingdom.

SOURCE: INFECTION AND IMMUNITY, (2000 Mar) 68 (3) 1109-15.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000327
Last Updated on STN: 20000327
Entered Medline: 20000316

AB A previously described attenuated TnphoA **mutant** (BRD441) of Salmonella enterica serovar Typhimurium C5 (I. Miller, D. Maskell, C. Hormaeche, K. Johnson, D. Pickard, and G. Dougan, Infect. Immun. 57:2758-2763, 1989) was characterized, and the transposon was shown to be inserted in **surA**, a gene which encodes a peptidylprolyl-cis, trans-isomerase. A defined **surA** deletion **mutation** was introduced into S. enterica serovar Typhimurium C5 and the **mutant** strain, named S. enterica serovar Typhimurium BRD1115, was extensively characterized both in vitro and in vivo. S. enterica serovar Typhimurium BRD1115 was found to be defective in the ability to adhere to and invade eukaryotic cells. Furthermore, S. enterica serovar Typhimurium BRD1115 was attenuated by at least 3 log units when administered orally or intravenously to BALB/c mice. Complementation of the **mutation** with a plasmid carrying the intact **surA** gene almost completely restored the virulence of BRD1115. In addition, S. enterica serovar Typhimurium BRD1115 demonstrated potential as a **vaccine** candidate, since mice **immunized** with BRD1115 were protected against subsequent challenge with S. enterica serovar Typhimurium C5. S. enterica serovar Typhimurium BRD1115 also showed potential as a vehicle for the effective delivery of heterologous antigens, such as the nontoxic, protective fragment C domain of tetanus toxin, to the murine immune system.

L18 ANSWER 10 OF 59 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2000263775 MEDLINE
DOCUMENT NUMBER: 20263775 PubMed ID: 10802185
TITLE: Investigation into the role of the serine protease HtrA in **Yersinia** pestis pathogenesis.
AUTHOR: Williams K; Oyston P C; Dorrell N; Li S; Titball R W; Wren B W
CORPORATE SOURCE: Pathogen Molecular Biology and Biochemistry Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK.
SOURCE: FEMS MICROBIOLOGY LETTERS, (2000 May 15) 186 (2) 281-6.
Journal code: FML; 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000720
Last Updated on STN: 20000720
Entered Medline: 20000711

AB The **HtrA** stress response protein has been shown to play a role in the virulence of a number of pathogens. For some organisms, **htrA mutants** are attenuated in the animal model and can be used as live **vaccines**. A **Yersinia** pestis **htrA** orthologue was identified, cloned and sequenced, showing 86% and 87% similarity to Escherichia coli and Salmonella typhimurium **HtrAs**. An isogenic Y. pestis **htrA mutant** was constructed using a reverse genetics approach. In contrast to the wild-type strain, the **mutant** failed to grow at an elevated temperature of 39 degrees C, but showed only a small increase in sensitivity to

oxidative stress and was only partially attenuated in the animal model. However, the **mutant** exhibited a different protein expression profile to that of the wild-type strain when grown at 28 degrees C to simulate growth in the flea.

L18 ANSWER 11 OF 59 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2000193538 MEDLINE
DOCUMENT NUMBER: 20193538 PubMed ID: 10727882
TITLE: Kinetics of the mucosal antibody secreting cell response and evidence of specific lymphocyte migration to the lung after oral **immunisation** with attenuated S. enterica var. typhimurium.
AUTHOR: Allen J S; Dougan G; Strugnell R A
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Melbourne, Parkville, Australia.
SOURCE: FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, (2000 Apr) 27 (4) 275-81.
Journal code: BP1; 9315554. ISSN: 0928-8244.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000518
Last Updated on STN: 20000518
Entered Medline: 20000511

AB The kinetic of mucosal secretory responses elicited by the **vaccine** vector Salmonella enterica var. typhimurium (S. typhimurium) was examined by enzyme linked immunospot (ELISPOT) and compared with serum responses. Mice **immunised** orally with BRD509, the aroA, **aroD mutant** of virulent S. typhimurium SL1344 expressing the C Fragment of tetanus toxin (TT), simultaneously developed an IgA antibody secreting cells (ASC) response in the gastrointestinal lamina propria, the spleen and the lung, against both S. typhimurium lipopolysaccharide (LPS) and TT. The magnitude of the ASC response was greatest in the gut, was boosted by a secondary **immunisation** at day 25, and the kinetic of the response did not correlate with the appearance of serum antibodies. This study suggests that S. typhimurium can engage the common mucosal immune system to effect mucosal secretory responses at distal sites, however, the magnitude of the responses is both greatest in the gut and antigen-specific. The ASC origin of the serum antibodies specific for S. typhimurium and antigens expressed by the **bacterium** is yet to be elucidated.

L18 ANSWER 12 OF 59 MEDLINE
ACCESSION NUMBER: 2001232830 MEDLINE
DOCUMENT NUMBER: 21082009 PubMed ID: 11214237
TITLE: Properties of recombinant HtrA: an otitis media **vaccine** candidate antigen from non-typeable **Haemophilus** influenzae.
AUTHOR: Cates G A; Yang Y P; Klyushnichenko V; Oomen R; Loosmore S M
CORPORATE SOURCE: Aventis Pasteur, Toronto, Ontario, Canada.
SOURCE: DEVELOPMENTS IN BIOLOGICALS, (2000) 103 201-4.
Journal code: DMO; 100940058. ISSN: 1424-6074.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20010517
Entered Medline: 20010503

AB Non-encapsulated or non-typable **Haemophilus** influenzae (NTHi) is a major cause of middle ear infections in young children. **HtrA** has been identified as a **vaccine** candidate antigen from NTHi; therefore physicochemical characterization of this antigen is important for **vaccine** development. Recombinant NTHi **HtrA** has been expressed in E. coli and shown to have serine protease activity. Several **mutant**, recombinant **HtrA** proteins were expressed and purified to obtain suitable **vaccine** antigens lacking protease activity. Two **mutants** with alterations at the putative active site His91 and Ser197, designated H91A and S197A were examined by circular dichroic spectropolarimetry (CD) to evaluate secondary structure. The S197A **mutant** had a more random secondary structure compared to wild-type rHtrA or H91A. It is likely that improper folding of S197A accounts for its lack of immunoprotective properties in a chinchilla model of otitis media.

L18 ANSWER 13 OF 59 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2000:767654 SCISEARCH
THE GENUINE ARTICLE: 360FW
TITLE: Attenuation and immunogenicity of a **Brucella** abortus **htrA** cycL double **mutant** in cattle
AUTHOR: Edmonds M; Booth N; Hagius S; Walker J; Enright F; Roop R M; Elzer P (Reprint)
CORPORATE SOURCE: LOUISIANA STATE UNIV, SCH VET MED, DEPT VET MICROBIOL & PARASITOL, BATON ROUGE, LA 70803 (Reprint); LOUISIANA STATE UNIV, SCH VET MED, DEPT VET MICROBIOL & PARASITOL, BATON ROUGE, LA 70803; LOUISIANA STATE UNIV, AGCTR, DEPT VET SCI, BATON ROUGE, LA 70803; LOUISIANA STATE UNIV, HLTH SCI CTR, DEPT MICROBIOL & IMMUNOL, SHREVEPORT, LA 71130
COUNTRY OF AUTHOR: USA
SOURCE: VETERINARY MICROBIOLOGY, (15 SEP 2000) Vol. 76, No. 1, pp. 81-90.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0378-1135.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB PHE1 is a **htrA** cycL double gene deletion **mutant** of virulent **Brucella** abortus strain 2308 (S2308) which has previously been evaluated in the murine and caprine models of bovine brucellosis. This report describes the results of studies conducted with this **mutant** in the natural bovine host. Six sexually mature, non-gravid heifers were inoculated via the conjunctival sac with 1x10(10) colony forming units (CFU) of either the parental S2308 or the **htrA** cycL gene deletion **mutant**, PHE1. At 4, 7 and 11 days post-inoculation. PHE1 was found to

colonize the bovine host at lower levels than S2308. In a second experiment. eight heifers in mid-gestation were infected with 1x10(7) CFU of either strain via the conjunctival sac. The virulent S2308 caused abortions or weak calves in 4/4 cows, while all four cows infected with PHE1 had healthy calves. Furthermore, PHE1 exhibited decreased resistance to killing by cultured bovine neutrophils and macrophages compared to the parental strain. These studies demonstrate that the B. abortus **htrA** cycL gene deletion **mutant** PHE1 is highly attenuated in the bovine host when compared to the virulent parental S2308.

L18 ANSWER 14 OF 59 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 2000484693 MEDLINE
DOCUMENT NUMBER: 20385108 PubMed ID: 10924789
TITLE: IEM101, a naturally attenuated **Vibrio** cholerae strain as carrier for genetically detoxified derivatives of cholera toxin.
AUTHOR: Fontana M R; Monaci E; Yanqing L; Guoming Q; Duan G; Rappuoli R; Pizza M
CORPORATE SOURCE: IRIS, Chiron S.p.A, Via Fiorentina 1, 53100, Siena, Italy.
SOURCE: VACCINE, (2000 Aug 15) 19 (1) 75-85.
Journal code: X6O; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001019
Last Updated on STN: 20001019
Entered Medline: 20001011

AB Two **mutants** of cholera toxin (CTS106 containing a Pro106-->Ser substitution and CTK63 containing a Ser63-->Lys substitution) with greatly reduced or no toxicity respectively, were expressed in the naturally attenuated IEM101 **Vibrio** cholerae strain (El Tor, Ogawa) which does not express cholera toxin (CT). Expression was driven by the natural promoter of CT, or by a promoter known to induce strong in vivo expression such as **nirB**. In the rabbit ileal loop assay, where 10(4) wild type **bacteria** were sufficient to induce fluid accumulation, 10(9) IEM101 expressing CTS106 **bacteria** were needed to induce some fluid accumulation, while IEM101 expressing CTK63 was inactive, even when 10(10) cells were used. When used to **immunize** mice intranasally, all **bacteria** induced vibriocidal antibodies; however, anti-CT antibodies were not induced by **bacteria** expressing low levels of CTK63 under the control of the ct promoter. Anti-CT antibodies were successfully induced by **bacteria** expressing high levels of CTK63 under the control of the **nirB** promoter, or by **bacteria** expressing low levels of CTS106. These data show that antibodies against cholera toxin can be induced in vivo by high level expression of a non toxic **mutant**, or by using a **mutant** with residual ADP-ribosyltransferase activity.In conclusion, we have shown that IEM101, a naturally attenuated **Vibrio** strain known to be safe and immunogenic in humans, can be engineered to express immunogenic levels of CTK63, and may represent a good candidate for **vaccination** against cholera.

L18 ANSWER 15 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:290239 BIOSIS
DOCUMENT NUMBER: PREV200000290239
TITLE: **Vaccines** containing **bacteria** attenuated by **mutation** of the **htrA** gene.
AUTHOR(S): Dougan, Gordan (1); Charles, Ian George; Hormaeche, Carlos Estenio; Johnson, Kevin Stuart; Chatfield, Steven Neville
CORPORATE SOURCE: (1) Beckenham UK
ASSIGNEE: Glaxo Wellcome Inc., Research Triangle Park, NC, USA
PATENT INFORMATION: US 5980907 November 09, 1999
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 9, 1999) Vol. 1228, No. 2, pp. No pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB Attenuated microorganism for use in immunoprophylaxis in which the attenuation is brought about by the presence of a mutation in the DNA sequence of the microorganism which encodes, or which regulates the expression of DNA encoding a protein that is produced in response to environmental stress, the microorganism optionally being capable of expressing DNA encoding a heterologous antigen.

L18 ANSWER 16 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-385492 [32] WPIDS
DOC. NO. CPI: C1999-113421
TITLE: **SurA** attenuated **mutant bacteria**.
DERWENT CLASS: B04 D16
INVENTOR(S): CHATFIELD, S N; DOUGAN, G; SYDENHAM, M
PATENT ASSIGNEE(S): (MEDE-N) MEDEVA EURO LTD
COUNTRY COUNT: 85
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9929342	A1	19990617	(199932)*	EN	53
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9914960	A	19990628	(199946)		
EP 1037664	A1	20000927	(200048)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
AU 739191	B	20011004	(200166)		
JP 2001525375	W	20011211	(200204)		52

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

WO 9929342	A1	WO 1998-GB3680	19981210
AU 9914960	A	AU 1999-14960	19981210
EP 1037664	A1	EP 1998-959023	19981210
		WO 1998-GB3680	19981210
AU 739191	B	AU 1999-14960	19981210
JP 2001525375	W	WO 1998-GB3680	19981210
		JP 2000-524011	19981210

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9914960	A	Based on	WO 9929342
EP 1037664	A1	Based on	WO 9929342
AU 739191	B	Previous Publ.	AU 9914960
		Based on	WO 9929342
JP 2001525375	W	Based on	WO 9929342

PRIORITY APPLN. INFO: GB 1997-26233 19971211
AN 1999-385492 [32] WPIDS
AB WO 9929342 A UPAB: 19990813
NOVELTY - A **bacterium** (I) attenuated by a non-reverting mutation in a gene encoding a protein which promotes folding of extracytoplasmic proteins for use in **vaccinating** a human or animal, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for **vaccine** comprising a pharmaceutically acceptable carrier or diluent and (I).

ACTIVITY - Antibacterial; Immunoprotective.

MECHANISM OF ACTION - **Vaccine**.

USE - The attenuated **bacterium** is useful as a multivalent **vaccine**. It can additionally act as a carrier for heterologous antigens such as fragment C of tetanus toxin. The surA gene causes attenuation of virulent **bacteria**. (I) can be administered to the host to raise an immune response.

Dwg.0/5

L18 ANSWER 17 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-337878 [28] WPIDS
DOC. NO. CPI: C1999-099389
TITLE: Attenuated RpoS-positive **bacteria** as immunogenic carriers.
DERWENT CLASS: B04 D16
INVENTOR(S): CURTISS, R; NICKERSON, C A
PATENT ASSIGNEE(S): (UNIW) UNIV WASHINGTON
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9925387	A1	19990527	(199928)*	EN	162
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9914595	A	19990607	(199943)		

US 6024961 A 20000215 (200016)
EP 1030690 A1 20000830 (200042) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
AU 736242 B 20010726 (200149)
KR 2001024650 A 20010326 (200161)
JP 2001523649 W 20011127 (200204) 153

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9925387	A1	WO 1998-US24295	19981113
AU 9914595	A	AU 1999-14595	19981113
US 6024961	A	US 1997-970789	19971114
EP 1030690	A1	EP 1998-958581	19981113
		WO 1998-US24295	19981113
AU 736242	B	AU 1999-14595	19981113
KR 2001024650	A	KR 2000-705297	20000515
JP 2001523649	W	WO 1998-US24295	19981113
		JP 2000-520820	19981113

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9914595	A	Based on	WO 9925387
EP 1030690	A1	Based on	WO 9925387
AU 736242	B	Previous Publ.	AU 9914595
		Based on	WO 9925387
JP 2001523649	W	Based on	WO 9925387

PRIORITY APPLN. INFO: US 1997-970789 19971114
AN 1999-337878 [28] WPIDS
AB WO 9925387 A UPAB: 19990719
NOVELTY - Genetically engineered cell (A) is a live attenuated **bacterium** having:
(1) an RpoS+ phenotype;
(2) a recombinant rpoS+ gene;
(3) one or more inactivating (attenuating) mutations, and
(4) recombinant gene encoding a desired gene product (I).
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) **vaccines** for human **immunization** containing (A) as a carrier microorganism;
(2) delivering (I) to a human using (A) or similar cells (A') that do not include the recombinant rpoS+ gene;
(3) production of (A) by selecting for RpoS+ phenotype, then introduction of attenuating mutations and the gene encoding (I);
(4) delivering (I) to a human by administering a live, attenuated strain of a **bacterium** (B) that has:
(i) a recombinant virulence gene, expressing a product that facilitates invasion and colonization of gut-asociated lymphoid tissue (GALT);
(ii) at least one attenuating mutation, and
(iii) gene encoding (I);
(5) (B); and
(6) assessing immunogenicity of **bacteria** by determining the RpoS phenotype (a positive phenotype indicating

higher immunogenicity than a negative one).

ACTIVITY - Antibacterial; antiviral; antifungal; antiparasitic; anti-allergic; contraceptive.

MECHANISM OF ACTION - Induction of a specific immune response.

USE - (A) are used to deliver a wide variety of (I), e.g. antigens, enzymes, immunoregulators or other pharmaceutically active compounds to humans, especially as **vaccines** to protect against many **bacterial**, fungal, viral or parasitic diseases, for desensitizing allergy patients and as contraceptives. They may also be used to deliver nucleic acid (released from the carrier cells after lysis) to target cells.

ADVANTAGE - Salmonella **vaccine** strains that are RpoS+ are more highly immunogenic than those with the RpoS- phenotype, providing a better balance between attenuation and immunogenicity. (A) can colonize, and deliver (I) to, lymphoid tissues in the gut, nose or other tissues.

Dwg.0/14

L18 ANSWER 18 OF 59 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 1999346230 MEDLINE
DOCUMENT NUMBER: 99346230 PubMed ID: 10417208
TITLE: Expression and immunogenicity of a **mutant** diphtheria toxin molecule, CRM(197), and its fragments in Salmonella typhi **vaccine** strain CVD 908-**htrA**.
AUTHOR: Orr N; Galen J E; Levine M M
CORPORATE SOURCE: Department of Pediatrics, Division of Infectious Diseases and Tropical Pediatrics, Center for Vaccine Development, Department of Medicine, Division of Geographic Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA.
CONTRACT NUMBER: RO1AI29471 (NIAID)
RO1AI40297 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1999 Aug) 67 (8) 4290-4.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990820
Last Updated on STN: 20000303
Entered Medline: 19990812

AB **Mutant** diphtheria toxin molecule CRM(197) and fragments thereof were expressed in attenuated Salmonella typhi CVD 908-**htrA**, and the constructs were tested for their ability to induce serum antitoxin. Initially, expressed proteins were insoluble, and the constructs failed to induce neutralizing antitoxin. Soluble CRM(197) was expressed at low levels by utilizing the hemolysin A secretion system from Escherichia coli.

L18 ANSWER 19 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:359414 BIOSIS
DOCUMENT NUMBER: PREV199900359414
TITLE: Salmonella typhi flagella are potent inducers of proinflammatory cytokine secretion by human monocytes.
AUTHOR(S): Wyant, Timothy L.; Tanner, Michael K.; Sztein,

Marcelo B. (1)
CORPORATE SOURCE: (1) Center for Vaccine Development, Departments of Pediatrics and Medicine, University of Maryland, 685 West Baltimore St., Rm. 480, Baltimore, MD, 21201 USA
SOURCE: Infection and Immunity, (July, 1999) Vol. 67, No. 7, pp. 3619-3624.
ISSN: 0019-9567.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The cytokine production patterns of human peripheral blood mononuclear cells (PBMC) in response to Salmonella typhi flagella (STF) were examined in culture supernatants of PBMC stimulated with STF. Consistent with previous findings in volunteers **vaccinated** with aroC **aroD** deletion **mutants** of S. typhi, PBMC from volunteers **immunized** with the licensed live Ty21a S. typhi **vaccine** secreted gamma interferon following exposure to STF. Stimulation with STF induced rapid de novo synthesis of tumor necrosis factor alpha (TNF-alpha) and interleukin-1beta (IL-1beta), followed by IL-6 and IL-10. Trypsin treatment of STF abrogated their effects, while polymyxin B had no effect. Intracellular cytokine measurements of STF-stimulated PBMC revealed the existence of monocyte subpopulations that produce only TNF-alpha, IL-1beta or both cytokines. Moreover, STF markedly decreased the percentage of CD14+ cells. These data demonstrate that STF are powerful monocyte activators which may have important implicationsfor **vaccine** development and for understanding the pathogenesis of S. typhi infection.

L18 ANSWER 20 OF 59 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 1999184971 MEDLINE
DOCUMENT NUMBER: 99184971 PubMed ID: 10084987
TITLE: The alternative sigma factor, sigmaE, is critically important for the virulence of Salmonella typhimurium.
AUTHOR: Humphreys S; Stevenson A; Bacon A; Weinhardt A B; Roberts M
CORPORATE SOURCE: Department of Veterinary Pathology, Glasgow University Veterinary School, Glasgow G61 1QH, United Kingdom.
SOURCE: INFECTION AND IMMUNITY, (1999 Apr) 67 (4) 1560-8.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990426
AB In Escherichia coli, extracytoplasmic stress is partially controlled by the alternative sigma factor, RpoE (sigmaE). In response to environmental stress or alteration in the protein content of the cell envelope, sigmaE upregulates the expression of a number of genes, including **htrA**. It has been shown that **htrA** is required for intramacrophage survival and virulence in Salmonella typhimurium. To investigate whether sigmaE-regulated genes other than **htrA** are involved in salmonella virulence, we

inactivated the rpoE gene of S. typhimurium SL1344 by allelic exchange and compared the phenotype of the **mutant** (GVB311) in vitro and in vivo with its parent and an isogenic **htrA mutant** (BRD915). Unlike E. coli, sigmaE is not required for the growth and survival of S. typhimurium at high temperatures. However, GVB311 did display a defect in its ability to utilize carbon sources other than glucose. GVB311 was more sensitive to hydrogen peroxide, superoxide, and antimicrobial peptides than SL1344 and BRD915. Although able to invade both macrophage and epithelial cell lines normally, the rpoE **mutant** was defective in its ability to survive and proliferate in both cell lines. The effect of the rpoE **mutation** on the intracellular behavior of S. typhimurium was greater than that of the **htrA mutation**. Both GVB311 and BRD915 were highly attenuated in mice. Neither strain was able to kill mice via the oral route, and the 50% lethal dose (LD50) for both strains via the intravenous (i.v.) route was very high. The i.v. LD50s for SL1344, BRD915, and GVB311 were <10, 5.5 x 10(5), and 1.24 x 10(7) CFU, respectively. Growth in murine tissues after oral and i.v. inoculation was impaired for both the **htrA** and rpoE **mutant**, with the latter **mutant** being more severely affected. Neither **mutant** was able to translocate successfully from the Peyer's patches to other organs after oral infection or to proliferate in the liver and spleen after i.v. inoculation. However, the **htrA mutant** efficiently colonized the livers and spleens of mice infected i.v., but the rpoE **mutant** did not. Previous studies have shown that salmonella **htrA mutants** are excellent live **vaccines**. In contrast, oral **immunization** of mice with GVB311 was unable to protect any of the mice from oral challenge with SL1344. Furthermore, i.v. **immunization** with a large dose (approximately 10(6) CFU) of GVB311 protected less than half of the orally challenged mice. Thus, our results indicate that genes in the sigmaE regulon other than **htrA** play a critical role in the virulence and immunogenicity of S. typhimurium.

L18 ANSWER 21 OF 59 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 1999115546 MEDLINE
DOCUMENT NUMBER: 99115546 PubMed ID: 9916080
TITLE: Characterization of candidate live oral Salmonella typhi **vaccine** strains harboring defined **mutations** in aroA, aroC, and **htrA**.
AUTHOR: Lowe D C; Savidge T C; Pickard D; Eckmann L; Kagnoff M F; Dougan G; Chatfield S N
CORPORATE SOURCE: Department of Cellular Physiology, The Babraham Institute, Babraham, Cambridge CB2 4AT, Imperial College of Science, Technology and Medicine, London SW7 2AY, United Kingdom.
SOURCE: INFECTION AND IMMUNITY, (1999 Feb) 67 (2) 700-7. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 20000303
Entered Medline: 19990309

AB The properties of two candidate Salmonella typhi-based live oral typhoid **vaccine** strains, BRD691 (S. typhi Ty2 harboring **mutations** in aroA and aroC) and BRD1116 (S. typhi Ty2 harboring **mutations** in aroA, aroC, and **htrA**), were compared in a number of in vitro and in vivo assays. BRD1116 exhibited an increased susceptibility to oxidative stress compared with BRD691, but both strains were equally resistant to heat shock. Both strains showed a similar ability to invade Caco-2 and HT-29 epithelial cells and U937 macrophage-like cells, but BRD1116 was less efficient at surviving in epithelial cells than BRD691. BRD1116 and BRD691 were equally susceptible to intracellular killing within U937 cells. Similar findings were demonstrated in vivo, with BRD1116 being less able to survive and translocate to secondary sites of infection when inoculated into the lumen of human intestinal xenografts in SCID mice. However, translocation of BRD1116 to spleens and livers in SCID mice occurred as efficiently as that of BRD691 when inoculated intraperitonally. The ability of BRD1116 to increase the secretion of interleukin-8 following infection of HT-29 epithelial cells was comparable to that of BRD691. Therefore, loss of the **HtrA** protease in S. typhi does not seem to alter its ability to invade epithelial cells or macrophages or to induce proinflammatory cytokines such as IL-8 but significantly reduces intracellular survival in human intestinal epithelial cells in vitro and in vivo.

L18 ANSWER 22 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:124154 BIOSIS
DOCUMENT NUMBER: PREV200200124154
TITLE: **Vaccines** containing a salmonella **bacteria** attenuated by **mutation** of the **htrA** gene.
AUTHOR(S): Dougan, G.; Charles, I. G.; Hormaeche, C. E.; Johnson, K. S.; Chatfield, S. N.
CORPORATE SOURCE: Beckenham, United Kingdom
ASSIGNEE: GLAXO WELLCOME INC.
PATENT INFORMATION: US 5804194 Sept. 8, 1998
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Sept. 8, 1998) Vol. 1214, No. 2, pp. 1692.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

L18 ANSWER 23 OF 59 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 1998234013 MEDLINE
DOCUMENT NUMBER: 98234013 PubMed ID: 9573069
TITLE: Genetic control of immune response to recombinant antigens carried by an attenuated Salmonella typhimurium **vaccine** strain: Nramp1 influences T-helper subset responses and protection against leishmanial challenge.
AUTHOR: Soo S S; Villarreal-Ramos B; Anjam Khan C M; Hormaeche C E; Blackwell J M
CORPORATE SOURCE: Department of Pathology, University of Cambridge, United Kingdom.
SOURCE: INFECTION AND IMMUNITY, (1998 May) 66 (5) 1910-7. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980520
Last Updated on STN: 19980520
Entered Medline: 19980514

AB Attenuated strains of Salmonella typhimurium have been widely used as vehicles for delivery and expression of **vaccine** antigens in murine models of infectious disease. In mice, early **bacterial** replication following infection with S. typhimurium is controlled by the gene (Nramp1, formerly Ity/Lsh/Bcg) encoding the natural-resistance-associated macrophage protein (Nramp1). Nramp1 regulates macrophage activation and has multiple pleiotropic effects, including regulation of tumor necrosis factor alpha, interleukin 1beta (IL-1beta), and major histocompatibility complex class II molecules, all of which influence antigen processing and presentation. Nramp1 also has a direct effect on antigen processing, possibly by regulating the activity of proteases in the late endosomal compartment. Hence, there are multiple ways (regulation of **bacterial** load or recombinant antigen dose, class II molecule expression, costimulatory or adjuvant activity, and antigen processing) that Nramp1 might influence responses to recombinant salmonella **vaccines**. To test the hypothesis that Nramp1 influences responses to **vaccination**, congenic mouse strains have been used to analyze immune responses to recombinant antigens (tetanus toxoid antigen and leishmanial gp63) carried by live attenuated S. typhimurium aroA **aroD** **mutants**. Results show that congenic mice carrying the wild-type (S. typhimurium resistance) Nramp1 allele mount a predominantly T-helper-1 (IL-2 and gamma interferon) response to **vaccination** and show enhanced resolution of lesions following challenge infection with Leishmania major. In contrast, mice carrying **mutant** (S. typhimurium susceptibility) Nramp1 mount a T-helper-2 (immunoglobulin E and IL-4) response and show exacerbated lesion growth upon challenge.

L18 ANSWER 24 OF 59 MEDLINE
ACCESSION NUMBER: 1998230472 MEDLINE
DOCUMENT NUMBER: 98230472 PubMed ID: 9570545
TITLE: Protective effect on Leishmania major infection of migration inhibitory factor, TNF-alpha, and IFN-gamma administered orally via attenuated Salmonella typhimurium.
AUTHOR: Xu D; McSorley S J; Tetley L; Chatfield S; Dougan G; Chan W L; Satoskar A; David J R; Liew F Y
CORPORATE SOURCE: Department of Immunology, University of Glasgow, United Kingdom.
SOURCE: JOURNAL OF IMMUNOLOGY, (1998 Feb 1) 160 (3) 1285-9. Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980520
Last Updated on STN: 19990129
Entered Medline: 19980514

AB The genes encoding murine macrophage migration inhibitory factor (MIF), IL-2, IFN-gamma or TNF-alpha were cloned individually into an expression plasmid under the control of the inducible promoter **nirB** and transfected into the aroA- **aroD-** deletion **mutant** strain of Salmonella typhimurium (BRD509). These S. typhimurium derivatives (henceforward called constructs and termed GIDMIF, GIDIL2, GIDIFN and GIDTNF) expressed their respective cytokines in vitro under anaerobic conditions and stably colonized BALB/c mice up to 14 days after oral administration. The highly susceptible BALB/c mice that had received the constructs orally and that had been subsequently infected via the footpad with Leishmania major, developed significantly reduced disease compared with control mice administered the untransfected Salmonella strain (BRD509). Importantly, a combination of GIDMIF, GIDIFN, and GIDTNF administered orally after L. major infection was able to significantly limit lesion development and reduced parasite loads by up to three orders of magnitude. Spleen and lymph node cells of mice administered this combination expressed markedly higher levels of inducible nitric oxide synthase (iNOS) compared with those from mice receiving an equivalent dose of the control strain of Salmonella (BRD509). These data therefore demonstrate the feasibility of therapeutic treatment in an infectious disease model using cytokines delivered by attenuated Salmonella. The protective effect observed correlates with the induction of inducible nitric oxide synthase in vivo.

L18 ANSWER 25 OF 59 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 1998147697 MEDLINE
DOCUMENT NUMBER: 98147697 PubMed ID: 9488373
TITLE: The **Haemophilus** influenzae HtrA protein is a protective antigen.
AUTHOR: Loosmore S M; Yang Y P; Oomen R; Shortreed J M; Coleman D C; Klein M H
CORPORATE SOURCE: Pasteur Merieux Connaught Canada Research, North York, Ontario.. sloosmore@ca.pmc-vacc.com
SOURCE: INFECTION AND IMMUNITY, (1998 Mar) 66 (3) 899-906. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF018151; GENBANK-AF018152
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980319
Last Updated on STN: 20000303
Entered Medline: 19980312

AB The **htrA** gene from two strains of nontypeable **Haemophilus** influenzae has been cloned and sequenced, and the encoded approximately 46-kDa **HtrA** proteins were found to be highly conserved. H. influenzae **HtrA** has approximately 55% identity with the Escherichia coli and Salmonella typhimurium **HtrA** stress response proteins, and expression of the H. influenzae **htrA** gene was inducible by high temperature. Recombinant **HtrA** (rHtrA) was expressed from E. coli, and the purified protein was found to have serine protease activity. rHtrA was found to be very immunogenic and partially protective in both the passive infant rat model of bacteremia and the active chinchilla model of otitis media. Immunoblot analysis

indicated that **HtrA** is antigenically conserved in encapsulated and nontypeable H. influenzae species. Site-directed **mutagenesis** was performed on the **htrA** gene to ablate the endogenous serine protease activity of wild-type **HtrA**, and it was found that eight of nine recombinant **mutant** proteins had no measurable residual proteolytic activity. Two **mutant** proteins were tested in the animal protection models, and one, H91A, was found to be partially protective in both models. H91A **HtrA** may be a good candidate antigen for a **vaccine** against invasive H. influenzae type b disease and otitis media and is currently in phase I clinical trials.

L18 ANSWER 26 OF 59 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 1998114379 MEDLINE
DOCUMENT NUMBER: 98114379 PubMed ID: 9453634
TITLE: Comparison of the abilities of different attenuated Salmonella typhimurium strains to elicit humoral immune responses against a heterologous antigen.
AUTHOR: Dunstan S J; Simmons C P; Strugnell R A
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Melbourne, Parkville, Victoria, Australia.. s.dunstan@pgrad.unimelb.edu.au
SOURCE: INFECTION AND IMMUNITY, (1998 Feb) 66 (2) 732-40. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980212

AB We compared the abilities of different Salmonella enterica var. Typhimurium (S. typhimurium) strains harboring **mutations** in the genes aroA, aroAD, purA, ompR, **htrA**, and cya crp to present the heterologous antigen, C fragment of tetanus toxin, to the mouse immune system. Plasmid pTETtac4, encoding C fragment, was transferred into the various S. typhimurium **mutants**, and the levels of antigen expression were found to be equivalent. After primary oral **immunization** of BALB/c mice, all attenuated strains were capable of penetrating the gut epithelium and colonizing the Peyer's patches and spleens of mice. Of all strains compared, the delta purA **mutant** colonized and persisted in the Peyer's patches at the lowest level, whereas the delta **htrA mutant** colonized and persisted in the spleen at the lowest level. The level of specific antibody elicited by the different strains against either S. typhimurium lipopolysaccharide or tetanus toxoid was strain dependent and did not directly correlate to the **mutants'** ability to colonize the spleen. The level of immunoglobulin G1 (IgG1) and IgG2a antibody specific for tetanus toxoid was determined in mice **immunized** with four S. typhimurium **mutants**. The level of antigen-specific IgG1 and IgG2a was significantly lower in animals **immunized** with S. typhimurium delta purA. Antigen-specific T-cell proliferation assays indicated a degree of variability in the capacity of some strains to elicit T cells to the heterologous antigen. Cytokine profiles (gamma interferon and interleukin-5)

revealed that the four S. typhimurium **mutants** tested induced a Th1-type immune response. Mice were challenged with a lethal dose of tetanus toxin 96 days after oral **immunization**. With the exception of the S. typhimurium delta purA **mutant**, all strains elicited a protective immune response. These data indicate that the level of total Ig specific for the carried antigen, C fragment, does not correlate with the relative invasiveness of the vector, but it is determined by the carrier **mutation** and the background of the S. typhimurium strain.

L18 ANSWER 27 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 15
ACCESSION NUMBER: 1998:122652 BIOSIS
DOCUMENT NUMBER: PREV199800122652
TITLE: Intracellular multiplication and virulence of Shigella flexneri auxotrophic mutants.
AUTHOR(S): Cersini, Antonella; Salvia, Anna Maria; Bernardini, Maria Lina (1)
CORPORATE SOURCE: (1) Dipartimento Biol. Cellulare Sviluppo, Fondazione Istituto Pasteur-Cenci Bolognetti, Univ. Roma "La Sapienza," via degli Apuli 1, 00185 Rome Italy
SOURCE: Infection and Immunity, (Feb., 1998) Vol. 66, No. 2, pp. 549-557.
ISSN: 0019-9567.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We have constructed and analyzed a group of Shigella flexneri 5 auxotrophic **mutants**. The wild-type strain M90T was **mutagenized** in genes encoding enzymes involved in the synthesis of (i) aromatic amino acids, (ii) nucleotides, and (iii) diaminopimelic acid. In this way, strains with single (aroB, aroC, **aroD**, purE, thyA, and dapB) and double (purE aroB, purE aroC, purE **aroD**, purE thyA) **mutations** were obtained. Although the Aro **mutants** had the same nutritional requirements when grown in laboratory media, they showed different degrees of virulence in vitro and in vivo. The aroB **mutant** was not significantly attenuated, whereas both the aroC and **aroD** strains were severely attenuated. p-Aminobenzoic acid (PABA) appeared to be the main requirement for the Aro **mutants'** growth in tissue culture. Concerning nucleotides, thymine reduced the pathogenicity, whereas adenine did not. However, when combined with another virulence-affecting **mutation**, adenine auxotrophy appeared to potentiate that **mutation's** effects. Consequently, the association of either the purE and aroC or the purE and **aroD mutations** had a great effect on virulence as measured by the Sereny test, whereas the purE aroB double **mutation** appeared to have only a small effect. All **mutants** except the dapB strain seemed to move within a Caco-2 cell monolayer after 3 h of infection. Nevertheless, the auxotrophs showing a high intracellular generation time were negative in the plaque assay. Knowledge of each **mutation's** role in attenuating Shigella strains will provide useful tools in designing **vaccine** candidates.

L18 ANSWER 28 OF 59 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 97230342 MEDLINE
DOCUMENT NUMBER: 97230342 PubMed ID: 9119506
TITLE: Salmonella typhimurium aroA, **htrA**, and

aroD htrA mutants cause progressive infections in athymic (nu/nu) BALB/c mice.

AUTHOR: Sinha K; Mastroeni P; Harrison J; de Hormaeche R D; Hormaeche C E

CORPORATE SOURCE: Department of Microbiology, The Medical School, University of Newcastle, Newcastle upon Tyne, United Kingdom.

SOURCE: INFECTION AND IMMUNITY, (1997 Apr) 65 (4) 1566-9. Journal code: GO7; 0246127. ISSN: 0019-9567.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199704

ENTRY DATE: Entered STN: 19970506
Last Updated on STN: 20000303
Entered Medline: 19970424

AB Athymic (nu/nu) BALB/c mice and their euthymic (nu/+) littermates were inoculated intravenously with live attenuated **vaccine** strains of Salmonella typhimurium. All strains caused progressive infections in the athymic mice but not in their euthymic littermates. Athymic mice given strain SL3261, an aroA derivative of SL1344, in doses between log 4.7 and 5.7 CFU were all severely ill and were killed by weeks 4 to 5. Athymic mice given log 4.7 CFU of a derivative of S. typhimurium C5 carrying a **mutation** in **htrA**, encoding a stress protein, were ill and were killed by week 7 in one experiment but survived to week 13 in another. Athymic mice given log 4.6 CFU of a C5 **aroD htrA** double **mutant** were ill and were killed at week 7. Athymic mice given SL3261 had high **bacterial** counts in the reticuloendothelial system at 4 weeks. Athymic mice given SL3261 or C5 **htrA** made immunoglobulin G3 (IgG3) (and to a lesser extent IgM) antibody to lipopolysaccharide (LPS), whereas euthymic mice made IgM, IgG1, IgG2a, IgG2b, and IgG3 anti-LPS antibodies. The results indicate that both aroA and **htrA** strains will produce slow, progressively lethal infections in athymic mice, that the **htrA** strain is more attenuated than the aroA strain as measured by time to death in this model, and that IgG3 anti-LPS antibody alone cannot suppress the progress of infections by very attenuated strains in athymic mice.

L18 ANSWER 29 OF 59 MEDLINE DUPLICATE 17

ACCESSION NUMBER: 1998065765 MEDLINE

DOCUMENT NUMBER: 98065765 PubMed ID: 9403507

TITLE: Cloning and characterisation of the aroA and aroD genes of Shigella dysenteriae type 1.

AUTHOR: Walker J C; Verma N K

CORPORATE SOURCE: Division of Biochemistry and Molecular Biology, Faculty of Science, School of Life Sciences, The Australian National University, Canberra.. John.Walker@anu.edu.au

SOURCE: MICROBIOLOGY AND IMMUNOLOGY, (1997) 41 (10) 809-13. Journal code: MX7; 7703966. ISSN: 0385-5600.

PUB. COUNTRY: Japan

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-U82268
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980206
Last Updated on STN: 19980206
Entered Medline: 19980127

AB The aroA and **aroD** genes from Shigella dysenteriae type 1, encoding 5-enolpyruvylshikimate 3-phosphate synthase and 3-dehydroquinase, respectively, were cloned by polymerase chain reaction (PCR). Their nucleotide sequences were determined and predicted to code for 46 kDa and 27.5 kDa proteins, respectively. Protein expressed from these genes using the minicell system, corresponded to the size of the predicted protein products. The cloned genes were shown to be functional by complementation of Escherichia coli aroA- and **aroD- mutants**. The predicted amino acid sequences of the cloned aroA (427 amino acids) and **aroD** (252 amino acids) genes of S. dysenteriae type 1 were found to be highly homologous to the corresponding genes in other **bacterial** species, indicating the high conservation of these housekeeping genes. The use of the cloned aroA and **aroD** genes in the development of a **vaccine** strain against S. dysenteriae is discussed.

L18 ANSWER 30 OF 59 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 1998043496 MEDLINE
DOCUMENT NUMBER: 98043496 PubMed ID: 9383148
TITLE: The HtrA family of serine proteases.
AUTHOR: Pallen M J; Wren B W
CORPORATE SOURCE: Department of Medical Microbiology, St Bartholomew's and the Royal London School of Medicine and Dentistry, UK.. m.pallen@qmw.ac.uk
SOURCE: MOLECULAR MICROBIOLOGY, (1997 Oct) 26 (2) 209-21.
Ref: 72
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 20000303
Entered Medline: 19980219

AB **HtrA**, also known as DegP and probably identical to the Do protease, is a heat shock-induced serine protease that is active in the periplasm of Escherichia coli. Homologues of **HtrA** have been described in a wide range of **bacteria** and in eukaryotes. Its chief role is to degrade misfolded proteins in the periplasm. Substrate recognition probably involves the recently described PDZ domains in the C-terminal half of **HtrA** and, we suspect, has much in common with the substrate recognition system of the tail-specific protease, Prc (which also possesses a PDZ domain). The expression of **htrA** is regulated by a complex set of signal transduction pathways, which includes an alternative sigma factor, RpoE, an anti-sigma factor, RseA, a two-component regulatory system, CpxRA, and two phosphoprotein phosphatases, PrpA and PrpB. **Mutations** in the **htrA** genes of Salmonella, **Brucella** and **Yersinia** cause

decreased survival in mice and/or macrophages, and **htrA mutants** can act as **vaccines**, as cloning hosts and as carriers of heterologous antigens.

L18 ANSWER 31 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:66359 BIOSIS
DOCUMENT NUMBER: PREV199799365562
TITLE: **Vaccine** efficacy of Salmonella strains expressing glycoprotein 63 with different promoters.
AUTHOR(S): McSorley, Stephen J.; Xu, Damo; Liew, F. Y. (1)
CORPORATE SOURCE: (1) Dep. Immunol., Univ. Glasgow, Glasgow G11 6NT UK
SOURCE: Infection and Immunity, (1997) Vol. 65, No. 1, pp. 171-178.
ISSN: 0019-9567.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The development of Salmonella **vaccine** vectors has been hindered by both the requirement for multiple doses to induce immune responses and a lack of plasmid stability. Direct comparisons of different promoter systems with the same antigen are necessary to address these important issues. We have previously described an AroA- **AroD-** deletion **mutant** of Salmonella typhimurium (GID101) which expresses the gene encoding the Leishmania major promastigote surface glycoprotein gp63 (GID101). While this construct provided significant protection against L. major challenge to highly susceptible BALB/c mice, this required at least two oral doses. We report here the use of two different inducible promoters, the **nirB** and osmC promoters, to improve **vaccine** efficacy. These constructs (termed GID105 and GID106, respectively) expressed gp63 in vitro under inducible conditions and colonized BALB/c mice after oral administration. GID105 demonstrated greater plasmid stability in vitro and in vivo than did either GID106 or GID101, which expresses gp63 constitutively. Spleen and lymph node cells from mice **immunized** with a single oral dose of GID105 proliferated in vitro in response to L. major and secreted gamma interferon, whereas cells from mice given the other constructs did not. Mice **immunized** with a single oral dose of GID105 or GID106 developed significantly smaller lesions upon challenge with L. major, whereas mice administered GID101 did not. Mice administered GID105 also showed considerable resistance to Leishmania donovani infection. These data provide a direct comparison of promoter systems and demonstrate that the use of inducible promoters such as the **nirB** promoter allows a considerable improvement over the previous **vaccine** construct in terms of protection against infection.

L18 ANSWER 32 OF 59 MEDLINE DUPLICATE 19
ACCESSION NUMBER: 1998090966 MEDLINE
DOCUMENT NUMBER: 98090966 PubMed ID: 9429252
TITLE: A **Brucella** melitensis high-temperature-requirement A (**htrA**) deletion **mutant** is attenuated in goats and protects against abortion.
AUTHOR: Phillips R W; Elzer P H; Robertson G T; Hagius S D; Walker J V; Fatemi M B; Enright F M; Roop R M 2nd
CORPORATE SOURCE: Department of Microbiology and Immunology, Louisiana State University Medical Center, Shreveport 71130,

USA.
CONTRACT NUMBER: AI28867 (NIAID)
SOURCE: RESEARCH IN VETERINARY SCIENCE, (1997 Sep-Oct) 63 (2) 165-7.
Journal code: R7D; 0401300. ISSN: 0034-5288.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980306
Last Updated on STN: 20000303
Entered Medline: 19980224

AB It has been previously demonstrated that a **Brucella** melitensis high-temperature-requirement A (**htrA**) deletion **mutant** is more susceptible to oxidative killing in vitro than the parental strain and is attenuated in mice. To evaluate the contribution of the B melitensis **HtrA** protease to virulence in ruminants, the capacity of the B melitensis **htrA mutant** RWP5 to produce abortion in goats was compared to that of the virulent parental strain 16M. Experimental infection with strain 16M caused abortion in eight of 12 pregnant nannies, while none of the 12 nannies inoculated with RWP5 aborted. Furthermore, intramuscular injection of fetuses in utero with RWP5 led to colonisation of the fetus with subsequent colonisation of the nanny, but no abortion was observed. Nannies **vaccinated** with RWP5 showed complete protection against abortion when challenged with 16M during the third trimester of pregnancy. However, these animals were not protected from colonisation by 16M. The results presented here clearly indicate that the B melitensis **htrA** gene product contributes to pathogenesis in goats, but the utility of B melitensis **htrA mutants** as **vaccines** in this host appears to be limited.

L18 ANSWER 33 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:107156 BIOSIS
DOCUMENT NUMBER: PREV199800107156
TITLE: Mucosal **vaccination** against HSV using live attenuated Salmonella typhimurium.
AUTHOR(S): Bakhsh, O. S.; Hormaeche, C. E.; Hill, T. J.; Williams, N. A.
CORPORATE SOURCE: Dep. Pathol. and Microbiol., Univ. Bristol, Sch. Med. Sci., University Walk, Bristol BS8 1TD UK
SOURCE: Immunology, (Dec., 1997) Vol. 92, No. SUPPL. 1, pp. 53.
Meeting Info.: 5th Annual Congress of the British Society for Immunology Brighton, England, UK December 2-5, 1997 British Society for Immunology
. ISSN: 0019-2805.
DOCUMENT TYPE: Conference
LANGUAGE: English

L18 ANSWER 34 OF 59 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 96417858 MEDLINE
DOCUMENT NUMBER: 96417858 PubMed ID: 8820649
TITLE: A Salmonella typhimurium htrA live **vaccine** expressing multiple copies of a peptide comprising amino acids 8-23 of herpes simplex virus glycoprotein

D as a genetic fusion to tetanus toxin fragment C protects mice from herpes simplex virus infection.

AUTHOR: Chabalgoity J A; Khan C M; Nash A A; Hormaeche C E
CORPORATE SOURCE: Department of Microbiology, University of Newcastle, Newcastle upon Tyne, UK.

SOURCE: MOLECULAR MICROBIOLOGY, (1996 Feb) 19 (4) 791-801.
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 20000303
Entered Medline: 19961216

AB Multiple tandem copies of an immunogenic epitope comprising amino acids 8-23 of glycoprotein D of herpes simplex virus (HSV) were expressed as C-terminal fusions to tetanus toxin fragment C (TetC) in different Salmonella typhimurium live **vaccine** strains. Expression of the longer fusions was best in strains harbouring a lesion in **htrA**, a stress protein gene. SL3261, an aroA strain, did not effectively express the longer fusions. Mice **immunised** with an S. typhimurium C5 **htrA** **mutant** expressing fusions with two or four copies of the peptide made an antibody response to both the peptide and TetC, whereas constructs expressing one copy of the peptide only elicited antibody to TetC. A non-immunogenic octameric fusion underwent rearrangements in vivo resulting in a predominantly monomeric fusion. In contrast, the S. typhimurium SL3261 aroA **vaccine** expressing the TetC-tetrameric fusion did not elicit antibody to the peptide. Sera from mice **immunised** with a single dose of the dimer and tetramer fusions in the **htrA** strain neutralised HSV in vitro, and the mice were protected from HSV infection as measured by a reduction in virus load in the ear pinna. We have previously shown that mice **vaccinated** with salmonella expressing TetC are protected against tetanus toxin and virulent salmonella challenge. These results suggest that it may be possible to develop a multivalent **vaccine** against salmonellosis, tetanus and HSV.

L18 ANSWER 35 OF 59 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 97069712 MEDLINE
DOCUMENT NUMBER: 97069712 PubMed ID: 8912695
TITLE: Cloning, sequencing, expression, purification and preliminary characterization of a type II dehydroquinase from Helicobacter pylori.
AUTHOR: Bottomley J R; Clayton C L; Chalk P A; Kleanthous C
CORPORATE SOURCE: School of Biological Sciences, University of East Anglia, Norwhich, U.K.
SOURCE: BIOCHEMICAL JOURNAL, (1996 Oct 15) 319 (Pt 2) 559-65.
Journal code: 9YO; 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X98878
ENTRY MONTH: 199612

ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961226

AB A heat-stable dehydroquinase was purified to near homogeneity from a plate-grown suspension of the Gram-negative stomach pathogen Helicobacter pylori, and shown from both its subunit and native molecular masses to be a member of the type II family of dehydroquinases. This was confirmed by N-terminal amino acid sequence data. The gene encoding this activity was isolated following initial identification, by random sequencing of the H. pylori genome, of a 96 bp fragment, the translated sequence of which showed strong identity to a C-terminal region of other type II enzymes. Southern blot analysis of a cosmid library identified several potential clones, one of which complemented an Escherichia coli **aroD** point **mutant** strain deficient in host dehydroquinase. The gene encoding the H. pylori type II dehydroquinase (designated aroQ) was sequenced. The translated sequence was identical to the N-terminal sequence obtained directly from the purified protein, and showed strong identity to other members of the type II family of dehydroquinases. The enzyme was readily expressed in E. coli from a plasmid construct from which several milligrams of protein could be isolated, and the molecular mass of the protein was confirmed by electrospray MS. The aroQ gene in H. pylori may function in the central biosynthetic shikimate pathway of this **bacterium**, thus opening the way for the construction of attenuated strains as potential **vaccines** as well as offering a new target for selective enzyme inhibition.

L18 ANSWER 36 OF 59 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 96316340 MEDLINE
DOCUMENT NUMBER: 96316340 PubMed ID: 8734637
TITLE: In vitro and in vivo phenotypes resulting from deletion of the high temperature requirement A (htrA) gene from the bovine **vaccine** strain **Brucella** abortus S19.
AUTHOR: Robertson G T; Elzer P H; Roop R M 2nd
CORPORATE SOURCE: Department of Microbiology and Immunology, Louisiana State University Medical Center, Shreveport 71130-3932, USA.
CONTRACT NUMBER: AI 28867 (NIAID)
SOURCE: VETERINARY MICROBIOLOGY, (1996 Apr) 49 (3-4) 197-207. Journal code: XBW; 7705469. ISSN: 0378-1135.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19961008
Last Updated on STN: 19961008
Entered Medline: 19960920

AB An **htrA** deletion **mutant** was created in the bovine **vaccine** strain, B. abortus S19, by replacing the majority of the **htrA** gene with a kanamycin resistance gene. Antibiotic selection for a double crossover event yielded kanamycin-resistant, ampicillin-sensitive colonies confirmed by Southern and western blot analysis to be **HtrA** deficient. The B. abortus S19 **htrA mutant** was significantly more susceptible than the parental strain to killing by H2O2 (P <

0.001) and O(2)- generated by the redox cycling agent paraquat ($P < 0.05$) in disk sensitivity assays. Deletion of the **htrA** gene from S19 produced a bimodal effect on the spleen colonization profile of this strain in BALB/c mice. At one week post-infection, the B. abortus S19 **htrA mutant** colonized the spleens of experimentally infected BALB/c mice at significantly lower levels ($P < 0.01$) than the parental strain. Enhanced clearance ($P < 0.05$) was also observed at later timepoints, i.e. 4 and 7 weeks post infection, however at 2 and 3 weeks post infection, the **mutant** and parental strains colonized the mice at equivalent levels. The temporal development of specific delayed type hypersensitivity and antibody responses in BALB/c mice infected with the **mutant** or parental strain were equivalent. These results suggest that the **htrA** gene product contributes to successful host colonization by S19. However, deletion of this gene does not radically alter the overall, characteristic spleen colonization profile of this **vaccine** strain in the BALB/c mouse model, nor compromise the capacity of this strain to elicit **Brucella** cellular or humoral immune responses in this experimental host.

L18 ANSWER 37 OF 59 MEDLINE DUPLICATE 23
ACCESSION NUMBER: 96351471 MEDLINE
DOCUMENT NUMBER: 96351471 PubMed ID: 8717403
TITLE: Attenuated Salmonella as live oral **vaccines** against typhoid fever and as live vectors.
AUTHOR: Levine M M; Galen J; Barry E; Noriega F; Chatfield S; Sztein M; Dougan G; Tacket C
CORPORATE SOURCE: Center for Vaccine Development, University of Maryland School of Medicine, Baltimore 21201, USA.
SOURCE: JOURNAL OF BIOTECHNOLOGY, (1996 Jan 26) 44 (1-3) 193-6. Ref: 19
Journal code: AL6; 8411927. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961025
Last Updated on STN: 20000303
Entered Medline: 19961016

AB Attenuated Salmonella typhi **vaccine** strain CVD 908, which harbors deletion **mutations** in aroC and **aroD**, has been shown to be well-tolerated and highly immunogenic, eliciting impressive serum antibody, mucosal IgA and cell-mediated immune responses. A further derivative prepared by introducing a deletion in **htrA** (which encodes a heat-shock protein that also has activity as a serine protease in CVD 908 (Chatfield et al., unpublished data) resulted in CVD 908-**htrA**. In phase 1 clinical trials, CVD 908-**htrA** appears very attractive as a live oral **vaccine** candidate. Both CVD 908 and CVD 908-**htrA** are useful as live vector **vaccines** to deliver foreign antigens to the immune system. Conditions that enhance the expression and immunogenicity of foreign antigens carried by CVD 908 and CVD 908-**htrA** are being investigated.

L18 ANSWER 38 OF 59 MEDLINE DUPLICATE 24
ACCESSION NUMBER: 95394851 MEDLINE
DOCUMENT NUMBER: 95394851 PubMed ID: 7545156
TITLE: Expression of Shigella dysenteriae serotype 1 O-antigenic polysaccharide by Shigella flexneri aroD **vaccine** candidates and different S. flexneri serotypes.
AUTHOR: Falt I C; Schweda E K; Klee S; Singh M; Floderus E; Timmis K N; Lindberg A A
CORPORATE SOURCE: Department of Immunology, Microbiology, Pathology, and Infectious Diseases, Karolinska Institute, Huddinge, Sweden.
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 Sep) 177 (18) 5310-5. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951020
Last Updated on STN: 19960129
Entered Medline: 19951012

AB The potential utility of Shigella flexneri **aroD** **vaccine** candidates for the development of bi- or multivalent **vaccines** has been explored by the introduction of the genetic determinants rfp and rfb for heterologous O antigen polysaccharide from Shigella dysenteriae serotype 1. The serotype Y **vaccine** strain SFL124 expressed the heterologous antigen qualitatively and quantitatively well, qualitatively in the sense of the O antigen polysaccharide being correctly linked to the S. flexneri lipopolysaccharide R3 core oligosaccharide and quantitatively in the sense that typical yields were obtained, with ratios of homologous to heterologous O antigen being 4:1 for one construct and 1:1 for another. Moreover, both polysaccharide chains were shown to be linked to position O-4 of the subterminal D-glucose residue of the R3 core. In contrast to the hybrid serotype Y SFL124 derivatives, analogous derivatives of serotype 2a **vaccine** strain SFL1070 did not elaborate a complete heterologous O antigen. Such derivatives, and analogous derivatives of rough, O antigen-negative **mutants** of SFL1070, formed instead a hybrid lipopolysaccharide molecule consisting of the S. flexneri lipid A R3 core with a single repeat unit of the S. dysenteriae type 1 O antigen. Introduction of the determinants for the S. dysenteriae type 1 O antigen into a second serotype 2a strain and into strains representing other serotypes of S. flexneri, revealed the following for the expression of the heterologous O antigen: serotypes 1a, 1b, 2a, and 5a did not produce the heterologous O antigen, whereas serotypes 2b, 3a, 3b, 4a, 4b, 5b, and X did.

L18 ANSWER 39 OF 59 MEDLINE
ACCESSION NUMBER: 94341908 MEDLINE
DOCUMENT NUMBER: 94341908 PubMed ID: 8063417
TITLE: Characterization of defined ompR mutants of Salmonella typhi: ompR is involved in the regulation of Vi polysaccharide expression.
AUTHOR: Pickard D; Li J; Roberts M; Maskell D; Hone D; Levine M; Dougan G; Chatfield S
CORPORATE SOURCE: Department of Biochemistry, Imperial College of

Science, Technology and Medicine, London, United Kingdom.
SOURCE: INFECTION AND IMMUNITY, (1994 Sep) 62 (9) 3984-93. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X78270
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941005
Last Updated on STN: 19941005
Entered Medline: 19940921

AB The ompB operon, comprising the ompR and envZ genes, was cloned from a Salmonella typhi Ty2 cosmid bank and characterized by DNA sequence analysis. The S. typhi ompR and envZ genes contained open reading frames encoding proteins of 240 and 451 amino acids, respectively. Comparison with the Salmonella typhimurium OmpB protein sequences revealed 99.5% homology. The DNA sequence data were used to identify appropriate restriction sites for generating a defined deletion of 517 bp within the open reading frame of the ompR gene. This deletion was introduced by homologous recombination into the chromosomes of two S. typhi strains which already harbored defined deletions in both the aroC and **aroD** genes. The presence of the deletions within ompR was confirmed by Southern hybridization and sequencing of the DNA fragments surrounding the deleted regions by PCR. The S. typhi ompR **mutants** displayed a marked decrease in OmpC and OmpF porin expression as demonstrated by examination of outer membrane preparations. It was also found that S. typhi strains harboring the defined ompR deletions no longer agglutinated with Vi antiserum. However, when a functional ompB operon was introduced back into the S. typhi ompR **mutants**, either on a multicopy plasmid or as a single-copy chromosomal replacement, the Vi+ phenotype was restored. The levels of Vi synthesis were also found to be sensitive to different concentrations of sodium chloride present in the growth medium, although the levels of sensitivity varied between different isolates of S. typhi. It is therefore concluded that the ompR-envZ two component regulatory system plays an important role in the regulation of Vi polysaccharide synthesis in S. typhi and that one of the environmental signals for this regulation may be osmolarity.

L18 ANSWER 40 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:436458 BIOSIS
DOCUMENT NUMBER: PREV199497449458
TITLE: Adaptive acid tolerance response by Salmonella typhi and candidate live oral typhoid **vaccine** strains.
AUTHOR(S): Hone, David M. (1); Harris, Andrea M.; Levine, Myron M.
CORPORATE SOURCE: (1) Cent. Vaccine Dev., Div. Geographic Med., Sch. Med., Univ. Md., 10 South Pine St., Baltimore, MD 21201 USA
SOURCE: Vaccine, (1994) Vol. 12, No. 10, pp. 895-898. ISSN: 0264-410X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The data presented here demonstrate that Salmonella typhi is capable

of expressing an acid tolerance response (ATR) and that effective induction of this response (in nutrient-rich medium) occurs at pH 5.0 in anaerobic conditions. The candidate live oral S. typhi **vaccine** strains made by precise genetic methods and which carry auxotrophic **mutations** were CVD 906 (carries defined attenuating deletion **mutations**: DELTA-aroC, DELTA-**aroD**), CVD 908 (carries defined attenuating deletion **mutations**: DELTA-aroC, DELTA-**aroD**), 541Ty (carries attenuating deletion **mutations**: aroA, purA), and galE, Vi-negative (via) strain EX462. All generate an effective ATR. In contrast, nitrosoguanidine-derived live oral typhoid **vaccine** strain Ty21a only weakly expresses acid tolerance. This further demonstrates that the non-specific **mutagenesis** process used to produce Ty21a affects genetic loci outside the intended target genes for **mutagenesis**, galE and via, and further emphasizes the importance of using precise genetic techniques when developing live oral S. typhi **vaccines**.

L18 ANSWER 41 OF 59 MEDLINE DUPLICATE 25
ACCESSION NUMBER: 94041666 MEDLINE
DOCUMENT NUMBER: 94041666 PubMed ID: 8225611
TITLE: Salmonella typhimurium delta aroA delta **aroD** **mutants** expressing a foreign recombinant protein induce specific major histocompatibility complex class I-restricted cytotoxic T lymphocytes in mice.
AUTHOR: Turner S J; Carbone F R; Strugnell R A
CORPORATE SOURCE: Department of Microbiology, University of Melbourne, Parkville, Victoria, Australia.
SOURCE: INFECTION AND IMMUNITY, (1993 Dec) 61 (12) 5374-80. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19940117
Entered Medline: 19931222

AB Recombinant Salmonella typhimurium aroA **aroD** **mutants** which expressed ovalbumin were constructed. The two expression constructs used were based on either pUC18 or pBR322. The pBR322-based construct was more stable in vitro and in vivo than the pUC-based construct. Salmonellae containing the stable pBR322-based plasmid induced major histocompatibility complex (MHC) class I-restricted cytotoxic T lymphocytes (CTL), in contrast to salmonellae containing the pUC18-based expression construct. The priming of MHC class I-restricted CTL was increased by multiple **immunizations**. The study described in this report suggest that S. typhimurium delta aro **mutants** have the capacity to induce MHC class I-restricted CTL against carried antigens and that MHC class I-restricted CTL responses require stable in vivo expression of the target antigen. Further, the results indicate that the Salmonella typhi delta aro **mutants** currently undergoing evaluation in studies with humans may be good carriers of viral antigens with CTL determinants.

L18 ANSWER 42 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1992-331735 [40] WPIDS
CROSS REFERENCE: 1992-331734 [40]
DOC. NO. CPI: C1992-147534
TITLE: Attenuated **bacteria** expressing heterologous proteins - e.g. **Bordetella** pertussis P.69 protein, useful as a **vaccine** against infections caused by Salmonella, **Bordetella, Vibrio, Haemophilus** etc..
DERWENT CLASS: B04 C06 D16
INVENTOR(S): CHARLES, I G; CHATFIELD, S N; FAIRWEATHER, N F
PATENT ASSIGNEE(S): (WELL) WELLCOME FOUND LTD; (WELL) BURROUGHS WELLCOME CO; (GLAX) GLAXO WELLCOME INC
COUNTRY COUNT: 39
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9215689	A1	19920917	(199240)*	EN	23
RW: AT BE CH DE DK ES FR GB GR IT LU MC NL OA SE					
W: AT AU BB BG BR CA CH CS DE DK ES FI GB HU JP KP KR LK LU MG MN MW NL NO PL RO RU SD SE US					
AU 9213508	A	19921006	(199301)		
NO 9302423	A	19930702	(199341)		
FI 9303757	A	19930826	(199345)		
EP 574466	A1	19931222	(199351)	EN	
R: AT BE CH DE DK ES FR GB GR IT LI LU MC NL					
CZ 9301005	A3	19940119	(199410)		
SK 9300555	A3	19931006	(199420)		
JP 06505158	W	19940616	(199428)		10
HU 66833	T	19950130	(199510)		
AU 664360	B	19951116	(199602)		
US 5547664	A	19960820	(199639)		10
US 5683700	A	19971104	(199750)		10
EP 574466	B1	19990519	(199924)	EN	
R: AT BE CH DE DK ES FR GB GR IT LI LU MC NL SE					
CZ 285118	B6	19990512	(199925)		
DE 69229221	E	19990624	(199931)		
ES 2131069	T3	19990716	(199935)		
NO 309331	B1	20010115	(200106)		
KR 240182	B1	20000115	(200116)		
HU 219535	B	20010528	(200140)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9215689	A1	WO 1992-GB387	19920305
AU 9213508	A	AU 1992-13508	19920305
		WO 1992-GB387	19920305
NO 9302423	A	WO 1992-GB387	19920305
		NO 1993-2423	19930702
FI 9303757	A	WO 1992-GB387	19920305
		FI 1993-3757	19930826
EP 574466	A1	EP 1992-905914	19920305
		WO 1992-GB387	19920305
CZ 9301005	A3	CZ 1993-1005	19920305
SK 9300555	A3	SK 1993-555	19930514

JP 06505158	W		JP 1992-505563 19920305
HU 66833	T		WO 1992-GB387 19920305
AU 664360	B		WO 1992-GB387 19920305
US 5547664	A	Cont of	HU 1993-2492 19920305
		Cont of	AU 1992-13508 19920305
		Cont of	WO 1992-GB387 19920305
US 5683700	A	Cont of	US 1993-81361 19930630
		Cont of	US 1994-246773 19940520
		Cont of	US 1994-354776 19941212
		Div ex	WO 1992-GB387 19920305
EP 574466	B1		US 1993-81361 19930630
CZ 285118	B6		US 1994-246773 19940520
DE 69229221	E		US 1994-354776 19941212
			US 1995-469507 19950606
ES 2131069	T3		EP 1992-905914 19920305
NO 309331	B1		WO 1992-GB387 19920305
KR 240182	B1		WO 1992-GB387 19920305
HU 219535	B		CZ 1993-1005 19920305
			DE 1992-629221 19920305
			EP 1992-905914 19920305
			WO 1992-GB387 19920305
			EP 1992-905914 19920305
			WO 1992-GB387 19920305
			NO 1993-2423 19930702
			WO 1992-GB387 19920305
			KR 1993-702594 19930830
			WO 1992-GB387 19920305
			HU 1993-2492 19920305

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9213508	A	Based on	WO 9215689
EP 574466	A1	Based on	WO 9215689
JP 06505158	W	Based on	WO 9215689
HU 66833	T	Based on	WO 9215689
AU 664360	B	Previous Publ.	AU 9213508
		Based on	WO 9215689
US 5683700	A	Div ex	US 5547664
EP 574466	B1	Based on	WO 9215689
CZ 285118	B6	Previous Publ.	CZ 9301005
		Based on	WO 9215689
DE 69229221	E	Based on	EP 574466
		Based on	WO 9215689
ES 2131069	T3	Based on	EP 574466
NO 309331	B1	Previous Publ.	NO 9302423
HU 219535	B	Previous Publ.	HU 66833
		Based on	WO 9215689

PRIORITY APPLN. INFO: GB 1991-21208 19911004; GB 1991-4596 19910305

AN 1992-331735 [40] WPIDS

CR 1992-331734 [40]

AB WO 9215689 A UPAB: 20010719

An attenuated **bacterium** is capable of expressing a heterologous protein, the expression of the heterologous protein being under the control of a promoter whose activity is induced by

anaerobic conditions. Pref., the attenuated **bacterium** is an aroA, aroC, aroA, **aroD** or aroA, **aroE** **mutant**. The pref. promoter is the **nirB** promoter.

USE/ADVANTAGE - Using the attenuated **bacteria** stable expression of the heterologous protein can be obtd. in vivo. The heterologous protein can be an antigen such as P.69 protein from Bordetta pertussis or tetanus toxin fragment C and the attenuated **bacteria** can be used as a live **vaccine** against infections caused by eg. Salmonella, **Bordetella**, **Vibrio** or **Haemophilus**

Dwg.0/5

ABEQ US 5547664 A UPAB: 19961004

A **vaccine** comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an attenuated Salmonella **bacterium** which contains a nirB promoter operably linked to a DNA sequence encoding a heterologous protein.

Dwg.0/5

ABEQ US 5683700 A UPAB: 19971217

A method for prophylactically treating a host against infection by a microorganism, which method comprises administering to the host an attenuated Salmonella **bacterium** which contains a nirB promoter operably linked to a DNA sequence encoding a heterologous protein, wherein the heterologous protein is expressed in the host and induces in the host an immune response against the microorganism.

Dwg.0/5

L18 ANSWER 43 OF 59 MEDLINE

ACCESSION NUMBER: 92267630 MEDLINE

DOCUMENT NUMBER: 92267630 PubMed ID: 1587589

TITLE: Safety, immunogenicity, and efficacy in monkeys and humans of invasive Escherichia coli K-12 hybrid **vaccine** candidates expressing Shigella flexneri 2a somatic antigen.

AUTHOR: Kotloff K L; Herrington D A; Hale T L; Newland J W; Van De Verg L; Cogan J P; Snoy P J; Sadoff J C; Formal S B; Levine M M

CORPORATE SOURCE: Department of Pediatrics, University of Maryland School of Medicine, Baltimore 21201.

SOURCE: INFECTION AND IMMUNITY, (1992 Jun) 60 (6) 2218-24. Journal code: GO7; 0246127. ISSN: 0019-9567.

PUB. COUNTRY: United States

(CLINICAL TRIAL)

(CONTROLLED CLINICAL TRIAL)

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199206

ENTRY DATE: Entered STN: 19920710

Last Updated on STN: 19960129

Entered Medline: 19920623

AB A live, oral Shigella **vaccine**, constructed by transfer of the 140-MDa invasiveness plasmid from Shigella flexneri 5 and the chromosomal genes encoding the group- and type-specific O antigen of S. flexneri 2a to Escherichia coli K-12, was tested in humans. Designated EcSf2a-1, this **vaccine** produced adverse reactions (fever, diarrhea, or dysentery) in 4 (31%) of 13 subjects who ingested a single dose of 1.0 x 10(9) CFU, while at

better-tolerated doses (5.0 x 10(6) to 5.0 x 10(7) CFU), it provided no significant protection against challenge with S. flexneri 2a. A further-attenuated **aroD mutant** derivative, EcSf2a-2, was then tested. Rhesus monkeys that received EcSf2a-2 in three oral doses of ca. 1.5 x 10(11) CFU experienced no increase in gastrointestinal symptoms compared with a control group that received an E. coli K-12 placebo. Compared with controls, the **vaccinated** monkeys were protected against shigellosis after challenge with S. flexneri 2a (60% efficacy; P = 0.001). In humans, EcSf2a-2 was well tolerated at inocula ranging from 5.0 x 10(6) to 2.1 x 10(9) CFU. However, after a single dose of 2.5 x 10(9) CFU, 4 (17%) of 23 subjects experienced adverse reactions, including fever (3 subjects) and diarrhea (209 ml) (1 subject), and after a single dose of 1.8 x 10(10) CFU, 2 of 4 subjects developed dysentery. Recipients of three doses of 1.2 to 2.5 x 10(9) CFU had significant rises in serum antibody to lipopolysaccharide (61%) and invasiveness plasmid antigens (44%) and in gut-derived immunoglobulin A antibody-secreting cells specific for lipopolysaccharide (100%) and invasiveness plasmid antigens (60%). Despite its immunogenicity, the **vaccine** conferred only 36% protection against illness (fever, diarrhea, or dysentery) induced by experimental challenge (P = 0.17). These findings illustrate the use of an epithelial cell-invasive E. coli strain as a carrier for Shigella antigens. Future studies must explore dosing regimens that might optimize the protective effects of the **vaccine** while eliminating adverse clinical reactions.

L18 ANSWER 44 OF 59 MEDLINE
ACCESSION NUMBER: 92112316 MEDLINE
DOCUMENT NUMBER: 92112316 PubMed ID: 1730487
TITLE: Comparison of the safety and immunogenicity of delta aroC delta aroD and delta cya delta crp Salmonella typhi strains in adult volunteers.
AUTHOR: Tacket C O; Hone D M; Curtiss R 3rd; Kelly S M; Losonsky G; Guers L; Harris A M; Edelman R; Levine M M
CORPORATE SOURCE: Department of Medicine, University of Maryland School of Medicine, Baltimore 21201.
CONTRACT NUMBER: AI26186 (NIAID)
NO1 AI15096 (NIAID)
RO1 AI29471 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1992 Feb) 60 (2) 536-41.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
(CLINICAL TRIAL)
(CONTROLLED CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920308
Last Updated on STN: 19960129
Entered Medline: 19920218

AB Three attenuated Salmonella typhi strains have been constructed by introducing deletions in aroC and **aroD** or deletions in cya and crp into one of two wild-type parent strains, Ty2 or ISP1820. These **mutant** strains were designated CVD 906 (ISP1820 delta aroC delta **aroD**), CVD 908 (Ty2 delta aroC delta

aroD), and chi 3927 (Ty2 delta cya delta crp). Two studies were conducted with 36 healthy adult inpatient volunteers to determine in a double-blind fashion the safety and immunogenicity of approximately 5 x 10(4) and 5 x 10(5) CFU of each of these three **vaccine** candidates given as a single dose. No statistically significant difference in the incidence of reactions among **vaccinees** was observed. Fever (oral temperature greater than or equal to 38.2 degrees C) occurred in 2 of 12 volunteers who received CVD 906, in 0 of 12 who received CVD 908, and in 1 of 12 who received chi 3927. **Vaccine** bacteremia without symptoms occurred in 1 of 12 **vaccinees** who received CVD 906, in 0 of 12 who received CVD 908, and in 2 of 12 who received chi 3927. Overall, 19 (53%) of 36 **vaccinees** developed immunoglobulin G antibody to S. typhi lipopolysaccharide after **vaccination** , with no statistically significant differences in the rate of seroconversion among volunteers in the three groups. We conclude that defined **mutations** in the aromatic biosynthetic pathway and in the cyclic AMP global regulatory system attenuate S. typhi. **Mutant** strains CVD 906, CVD 908, and chi 3927 are highly (and approximately equally) immunogenic but possibly differ in their propensity to induce fever. Further studies are needed to document the apparent relative safety of CVD 908 as a typhoid **vaccine** and as a **vaccine** carrier of foreign antigens.

L18 ANSWER 45 OF 59 MEDLINE DUPLICATE 26
ACCESSION NUMBER: 92303181 MEDLINE
DOCUMENT NUMBER: 92303181 PubMed ID: 1609547
TITLE: Clinical acceptability and immunogenicity of CVD 908 Salmonella typhi **vaccine** strain.
AUTHOR: Tacket C O; Hone D M; Losonsky G A; Guers L; Edelman R; Levine M M
CORPORATE SOURCE: Department of Medicine, University of Maryland School of Medicine, Baltimore 21201.
CONTRACT NUMBER: NO1 A115096
RO1 A129471
SOURCE: VACCINE, (1992) 10 (7) 443-6.
Journal code: X60; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
(CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
(RANDOMIZED CONTROLLED TRIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920731
Last Updated on STN: 19980206
Entered Medline: 19920723

AB An attenuated Salmonella typhi strain has been sought as an improved oral typhoid **vaccine** and as a carrier of protective antigens of other pathogens to make hybrid **vaccines**. Ideally, such a strain would be safe and induce protective immune responses after a single oral dose. CVD 908 is a **mutant** of S. typhi wild-type strain Ty2 with recombinant deletions in two genes, aroC and **aroD**. In phase 1 testing to date, this strain has not produced febrile responses or other significant adverse reactions in adult volunteers given doses of 5 x 10(4) to 5 x 10(7) organisms with sodium bicarbonate. In addition, after just a

single oral dose of 5 x 10(7) colony-forming units, this strain induced IgG seroconversion to S. typhi lipopolysaccharide in 83% of **vaccinees** and stimulated specific IgA-secreting gut-derived lymphocytes in 100% of **vaccinees**. CVD 908 is a new oral typhoid **vaccine** that should be further investigated as a carrier for expressing foreign antigens in recombinant **vaccine** constructs.

L18 ANSWER 46 OF 59 MEDLINE DUPLICATE 27
ACCESSION NUMBER: 92286026 MEDLINE
DOCUMENT NUMBER: 92286026 PubMed ID: 1598787
TITLE: Live oral auxotrophic Shigella flexneri SFL124 **vaccine** with a deleted aroD gene: characterization and monkey protection studies.
AUTHOR: Karnell A; Stocker B A; Katakura S; Reinholt F P; Lindberg A A
CORPORATE SOURCE: Karolinska Institute, Department of Clinical Bacteriology, Huddinge Hospital, Sweden.
SOURCE: VACCINE, (1992) 10 (6) 389-94.
Journal code: X6O; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920717
Last Updated on STN: 19970203
Entered Medline: 19920709

AB Shigella flexneri SFL124, with a deletion encompassing all, or nearly all, of the coding sequence of gene **aroD** was obtained after selection on a fusaric acid medium supplemented with 2,3-dihydroxybenzoic acid for tetracycline-sensitive **mutants** of S. flexneri SFL114 which is an **aroD**::Tn10 transductant. Two of 20 tetracycline-sensitive **mutants** tested in colony hybridization with a 32P-labelled DNA probe of approximately 1400 base pairs (comprising all except the 75 N-terminal base pairs of the coding region of gene **aroD**) did not hybridize. The selected **mutant** SFL124 is Congo-red positive, invades and shows a limited multiplication in HeLa cells and does not cause keratoconjunctivitis in guinea-pigs. It is well tolerated by Macaca fascicularis monkeys, is excreted for up to 4 days, elicits a slight inflammatory reaction in the colonic mucosa, stimulates significant secretory IgA responses in the intestine and serum IgA and IgG responses against the S. flexneri cell envelope lipopolysaccharide. The immune response conferred a complete protection against challenge with 1 x 10(11) (equivalent to a 100 LD50 dose) live S. flexneri SFL1.

L18 ANSWER 47 OF 59 MEDLINE
ACCESSION NUMBER: 92334130 MEDLINE
DOCUMENT NUMBER: 92334130 PubMed ID: 1630300
TITLE: Impaired resistance to infection does not increase the virulence of Salmonella htrA live **vaccines** for mice.
AUTHOR: Strahan K; Chatfield S N; Tite J; Dougan G; Hormaeche C E
CORPORATE SOURCE: Department of Pathology, Cambridge, U.K.
SOURCE: MICROBIAL PATHOGENESIS, (1992 Apr) 12 (4) 311-7.

Journal code: MIC; 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920904
Last Updated on STN: 19970203
Entered Medline: 19920820

AB We have described a new class of live attenuated salmonella **vaccines** harbouring lesions in **htrA**, a stress protein gene previously. The virulence and invasiveness of Salmonella **htrA mutants** was investigated in three models of increased susceptibility to Salmonella infection. These included BALB/c mice, either given sublethal whole body irradiation (350 R) or administered rabbit anti-TNF alpha antiserum, and (CBA/NfemaleXBALB/cmale)F1 male mice which express the xid sex-linked B cell defect of CBA/N mice and are more susceptible to salmonellae than female littermates. Salmonella typhimurium **htrA mutants** derived from virulent strains, C5046 (C5 **htrA**::TnphoA) and BRD726 (SL1344 delta **htrA**) were not more invasive in immunosuppressed mice than in normal controls in the three mouse models of defective immunity. The results indicate that susceptibility to S. typhimurium **htrA vaccines** derived from virulent parents is not enhanced by conditions of impaired resistance to infection.

L18 ANSWER 48 OF 59 MEDLINE
ACCESSION NUMBER: 92261298 MEDLINE
DOCUMENT NUMBER: 92261298 PubMed ID: 1584006
TITLE: Evaluation of Salmonella typhimurium strains harbouring defined **mutations** in **htrA** and aroA in the murine salmonellosis model.
AUTHOR: Chatfield S N; Strahan K; Pickard D; Charles I G; Hormaeche C E; Dougan G
CORPORATE SOURCE: Vaccines Research Unit, Medeva Group Research, Wellcome Research Labs, Beckenham, Kent, U.K.
SOURCE: MICROBIAL PATHOGENESIS, (1992 Feb) 12 (2) 145-51.
Journal code: MIC; 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920626
Last Updated on STN: 19970203
Entered Medline: 19920618

AB Derivatives of the mouse-virulent Salmonella typhimurium strain SL1344 were constructed harbouring defined **mutations** in **htrA**, aroA or **htrA** aroA combined. When administered orally or intravenously to BALB/c mice, all the **mutants** were found to be highly attenuated. All **mutants** were able to confer significant protection against lethal challenge with SL1344 after a single oral dose of live organisms. SL1344 **htrA mutants** persisted in livers and spleens at a lower level than SL1344 aroA **mutants** after intravenous administration. SL1344 **htrA** aroA

mutants persisted at an even lower level and were cleared from the livers and spleens of mice within 21 days of intravenous administration. Thus **htrA** and **htrA** aroA **mutants** can be considered as potential oral **vaccines** against salmonellosis.

L18 ANSWER 49 OF 59 MEDLINE DUPLICATE 28
ACCESSION NUMBER: 92211328 MEDLINE
DOCUMENT NUMBER: 92211328 PubMed ID: 1348268
TITLE: Cloning of dapD, aroD and asd of Leptospira interrogans serovar icterohaemorrhagiae, and nucleotide sequence of the asd gene.
AUTHOR: Baril C; Richaud C; Fournie E; Baranton G; Saint Girons I
CORPORATE SOURCE: Unite de Bacteriologie Moleculaire et Medicale, Institut Pasteur, Paris, France.
SOURCE: JOURNAL OF GENERAL MICROBIOLOGY, (1992 Jan) 138 (Pt 1) 47-53.
Journal code: I87; 0375371. ISSN: 0022-1287.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M77500
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920515
Last Updated on STN: 19950206
Entered Medline: 19920507

AB Metabolites such as diaminopimelate and some aromatic derivatives, not synthesized in mammalian cells, are essential for growth of **bacteria**. As a first step towards the design of a new human live **vaccine** that uses attenuated strains of Leptospira interrogans, the asd, **aroD** and dapD genes, encoding aspartate beta-semialdehyde dehydrogenase, 3-dehydroquinase and tetrahydrodipicolinate N-succinyltransferase, respectively, were cloned by complementation of Escherichia coli **mutants**. The complete nucleotide sequence of the asd gene was determined and found to contain an open reading frame capable of encoding a protein of 349 amino acids with a calculated Mr of 38,007. Comparison of this deduced L. interrogans aspartate beta-semialdehyde dehydrogenase amino acid sequence with those of the same enzyme from Saccharomyces cerevisiae and Corynebacterium glutamicum revealed 46% and 36% identity, respectively. By contrast, the identity between the L. interrogans enzyme and the Streptococcus mutans or E. coli enzymes was less than 31%. Highly conserved sequences within aspartate semialdehyde dehydrogenase from the five organisms were observed at the amino and carboxyl termini, and around the cysteine of the active site.

L18 ANSWER 50 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1991-325215 [44] WPIDS
DOC. NO. CPI: C1991-140539
TITLE: Attenuated microorganism useful in live **vaccines** - attenuated by mutation in DNA sequence encoding e.g. a heat shock protein.
DERWENT CLASS: B04 D16
INVENTOR(S): CHARLES, I G; CHATFIELD, S N; DOUGAN, G; HORMAECHE, C E; JOHNSON, K S; HORMAECHE, C; CHARLES, I;

PATENT ASSIGNEE(S): JOHNSON, K; CHATFIELD, S
(WELL) WELLCOME FOUND LTD; (GLAX) GLAXO WELLCOME INC

COUNTRY COUNT: 25

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9115572	A	19911017	(199144)*		26
RW: AT BE CH DE DK ES FR GB GR IT LU NL SE					
W: AU CA HU JP KR US					
AU 9175417	A	19911030	(199205)		
ZA 9102397	A	19921125	(199301)		23
EP 524205	A1	19930127	(199304)	EN	26
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE					
TW 205567	A	19930511	(199337)		
JP 05507842	W	19931111	(199350)		11
NZ 237616	A	19940325	(199426)		
HU 65496	T	19940628	(199429)		
AU 659995	B	19950608	(199531)		
EP 524205	B1	19970827	(199739)	EN	19
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE					
DE 69127440	E	19971002	(199745)		
ES 2106776	T3	19971116	(199801)		
US 5804194	A	19980908	(199843)		
IL 97720	A	19990620	(199937)		
US 5980907	A	19991109	(199954)		
PH 29735	A	19960517	(200012)		
HU 217776	B	20000428	(200030)		
JP 3054440	B2	20000619	(200033)		11
KR 202771	B1	19990615	(200061)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
ZA 9102397	A		ZA 1991-2397	19910328
EP 524205	A1		EP 1991-906493	19910328
			WO 1991-GB484	19910328
TW 205567	A		TW 1991-102452	19910328
JP 05507842	W		JP 1991-506347	19910328
			WO 1991-GB484	19910328
NZ 237616	A		NZ 1991-237616	19910328
HU 65496	T		WO 1991-GB484	19910328
			HU 1992-3098	19910328
AU 659995	B		AU 1991-75417	19910328
EP 524205	B1		EP 1991-906493	19910328
			WO 1991-GB484	19910328
DE 69127440	E		DE 1991-627440	19910328
			EP 1991-906493	19910328
			WO 1991-GB484	19910328
ES 2106776	T3		EP 1991-906493	19910328
US 5804194	A	Cont of	WO 1991-GB484	19910328
		Cont of	US 1992-952737	19921130
		Cont of	US 1994-239910	19940509
			US 1994-350741	19941207
IL 97720	A		IL 1991-97720	19910328
US 5980907	A	Cont of	WO 1991-GB484	19910328

		Cont of	US 1992-952737	19921130
		Cont of	US 1994-239910	19940509
		Cont of	US 1994-350741	19941207
			US 1995-463875	19950605
PH 29735	A		PH 1991-42219	19910327
HU 217776	B		WO 1991-GB484	19910328
			HU 1992-3098	19910328
JP 3054440	B2		JP 1991-506347	19910328
			WO 1991-GB484	19910328
KR 202771	B1		KR 1992-702407	19920930

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 524205	A1	Based on	WO 9115572
JP 05507842	W	Based on	WO 9115572
HU 65496	T	Based on	WO 9115572
AU 659995	B	Previous Publ.	AU 9175417
		Based on	WO 9115572
EP 524205	B1	Based on	WO 9115572
DE 69127440	E	Based on	EP 524205
		Based on	WO 9115572
ES 2106776	T3	Based on	EP 524205
US 5980907	A	Cont of	US 5804194
HU 217776	B	Previous Publ.	HU 65496
		Based on	WO 9115572
JP 3054440	B2	Previous Publ.	JP 05507842
		Based on	WO 9115572

PRIORITY APPLN. INFO: GB 1990-7194 19900330

AN 1991-325215 [44] WPIDS

AB WO 9115572 A UPAB: 20000105

Microorganisms (I) for use in immunoprophylaxis are attenuated as a result of the presence of a mutation in the DNA sequence of the microorganism which encodes, or which regulates the expression of DNA encoding, a protein that is produced in response to environmental stress. The microorganism is opt. capable of expression DNA encoding a heterologous antigen. Also new is a **vaccine** contg. (I).

The protein is a nutrient deprivation protein, toxic stress protein, metabolic distress protein or especially a heat shock protein encoded by the htrA gene. The microorganism is a **bacterium** such as **Bordetella, Vibrio,** Haemopilus, Esherichia or especially Salmonella.

USE/ADVANTAGE - (I) are useful in live **vaccines** and immunoprophylaxis of e.g. salmonellosis, whooping cough, meningitis and gonorrhoea. Dosage of S. typti is 10 power 9 - 10 power 11 organisms/dose.

An attentuated form of S. typhimurium (strain 046) had log 10 ID50 of more than 9 cells, cf. the parental virulent strain C5 which had a log 10 LD50 of 6.38 cells, 28 days following oral administration.

Dwg.0/3

ABEQ ZA 9102397 A UPAB: 19930928

Microorganisms (I) for use in immunoprophylaxis are attenuated as a result of the presence of a mutation in the DNA sequence of the microorganism which encodes, or which regulates the expression of

DNA encoding, a protein that is produced in response to environmental stress. The microorganism is opt. capable of expression DNA encoding a heterologous antigen. Also new is a **vaccine** contg. (I).

The protein is a nutrient deprivation protein, toxic stress protein, metabolic distress protein or esp. a heat shock protein encoded by the htrA gene. The microorganism is a **bacterium** e.g. **Bordetella, Vibrio**, Haemopilus, Esherichia or especially Salmonella.

USE/ADVANTAGE - (I) are useful in live **vaccines** and immunoprophylaxis of e.g. salmonellosis, whooping cough, meningitis and gonorrhoea. Dosage of S. typti is 10 power(9)- 10 power (11) organisms/dose.

An attenuated form of S. typhimurium (strain 046) and log 10 ID50 of more than 9 cells, cf. the parental virulent strain C5 which had a log 10LD50 of 6.38 cells, 28 days following oral administration

ABEQ EP 524205 B UPAB: 19970926

A **vaccine** comprising a pharmaceutically acceptable carrier and an effective amount of a **bacterium** attenuated by a non-reverting **mutation** in the **htrA** gene.

Dwg.0/3

L18 ANSWER 51 OF 59 MEDLINE

ACCESSION NUMBER: 92101612 MEDLINE

DOCUMENT NUMBER: 92101612 PubMed ID: 1759503

TITLE: Construction of genetically defined double aro mutants of Salmonella typhi.

AUTHOR: Hone D M; Harris A M; Chatfield S; Dougan G; Levine M M

CORPORATE SOURCE: Department of Medicine, University of Maryland School of Medicine, University of Maryland, Baltimore 21201.

CONTRACT NUMBER: RO1-AI-29471 (NIAID)

SOURCE: VACCINE, (1991 Nov) 9 (11) 810-6.
Journal code: X6O; 8406899. ISSN: 0264-410X.

PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199202

ENTRY DATE: Entered STN: 19920223
Last Updated on STN: 19920223
Entered Medline: 19920206

AB The construction of genetically defined, double aro **mutant** strains CVD906 and CVD908, which were derived from Salmonella typhi strain ISP1820 (a recent isolate of S. typhi from Chile) and from laboratory strain Ty2, respectively, is described. Strains CVD906 and CVD908 differ from previously described aro **mutants** of S. typhi as their aro deletion **mutations** do not extend beyond the limits of the **mutated** aro genes, and no antibiotic-resistance genes, plasmid sequences or S. typhimurium DNA sequences remain in the **mutant** strains. In minimal medium the aro **mutants** of S. typhi are unable to replicate whereas the wild type parent strains grow well in minimal medium. Using intraperitoneal inoculation of mice with S. typhi strains suspended in hog gastric mucin as a virulence assay, it is shown that the single aro **mutants** and the double aro **mutants** of Ty2 and ISP1820 are attenuated in mice. Trans

complementation of the aro **mutants** with the aroC gene or **aroD** gene, or both, results in strains that are phenotypically identical to that of the wild type parents indicating that no measurable additional changes other than loss of the aro gene function occurred during strain construction.

L18 ANSWER 52 OF 59 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92046746 EMBASE
DOCUMENT NUMBER: 1992046746
TITLE: Comparison of the safety and immunogenicity of .DELTA.aroC .DELTA.aroD and .DELTA.cya .DELTA.crp Salmonella typhi strains in adult volunteers.
AUTHOR: Tacket C.O.; Hone D.M.; Curtiss III R.; Kelly S.M.; Losonsky G.; Guers L.; Harris A.M.; Edelman R.; Levine M.M.
CORPORATE SOURCE: Department of Medicine, Center for Vaccine Development, Maryland Univ. School/Medicine,Baltimore, MD 21201, United States
SOURCE: Infection and Immunity, (1991) 60/2 (536-541). ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
030 Pharmacology
037 Drug Literature Index
038 Adverse Reactions Titles
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Three attenuated Salmonella typhi strains have been constructed by introducing deletions in aroC and **aroD** or deletions in cya and crp into one of two wild-type parent strains, Ty2 or ISP1820. These **mutant** strains were designated CVD 906 (ISP1820 .DELTA.aroC .DELTA.**aroD**), CVD 908 (Ty2 .DELTA.aroC .DELTA. **aroD**), and AHp3927 (Ty2 .DELTA.cya .DELTA.crp). Two studies were conducted with 36 healthy adult inpatient volunteers to determine in a double-blind fashion the safety and immunogenicity of approximately 5 x 104 and 5 x 105 CFU of each of these three **vaccine** candidates given as a single dose. No statistically significant difference in the incidence of reactions among **vaccinees** was observed. Fever (oral temperature .gtoreq. 38.2.degree.C) occurred in 2 of 12 volunteers who received CVD 906, in 0 of 12 who received CVD 908, and in 1 of 12 who received AHp3927. **Vaccine** bacteremia without symptoms occurred in 1 of 12 **vaccinees** who received CVD 906, in 0 of 12 who received CVD 908, and in 2 of 12 who received AHp3927. Overall, 19 (53%) of 36 **vaccinees** developed immunoglobulin G antibody to S. typhi lipopolysaccharide after **vaccination**, with no statistically significant differences in the rate of seroconversion among volunteers in the three groups. We conclude that defined **mutations** in the aromatic biosynthetic pathway and in the cyclic AMP global regulatory system attenuate S. typhi. **Mutant** strains CVD 906, CVD 908, and AHp3927 are highly (and approximately equally) immunogenic but possibly differ in their propensity to induce fever. Further studies are needed to document the apparent relative safety of CVD 908 as a typhoid **vaccine** and as a **vaccine** carrier of foreign antigens.

L18 ANSWER 53 OF 59 MEDLINE DUPLICATE 29

ACCESSION NUMBER: 91181301 MEDLINE
DOCUMENT NUMBER: 91181301 PubMed ID: 2008797
TITLE: Oral **vaccination** of calves against experimental salmonellosis using a double aro mutant of Salmonella typhimurium.
AUTHOR: Jones P W; Dougan G; Hayward C; Mackensie N; Collins P; Chatfield S N
CORPORATE SOURCE: Karolinska Institute, Dept. Clinical Bacteriology, Huddinge Hospital, Sweden.
SOURCE: VACCINE, (1991 Jan) 9 (1) 29-34.
Journal code: X6O; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910519
Last Updated on STN: 19910519
Entered Medline: 19910501

AB An attenuated derivative of the fully calf virulent Salmonella typhimurium strain ST4/74 was constructed by introducing stable **mutations** into genes aroA and **aroD** rendering the strain dependent for growth on aromatic compounds. The strain (BRD562) was used to **vaccinate** orally ten Friesian calves 7 days after birth with approximately 10(10) live organisms. The only effects of **vaccination** in the calves was a transient faecal excretion of the organism, mild transient diarrhoea and mild pyrexia in one animal. Two of these animals were tested for the presence of BRD562 at necropsy 56 days after birth but none were shown to be infected. Eight of the **vaccinated** calves were subjected to an oral challenge infection with ST4/74 28 days after **vaccination** and seven out of the eight animals survived. The only effect of challenge in the surviving calves was mild pyrexia and transient excretion of ST4/74 in their faeces; only one of the seven animals was found to be infected at necropsy 28 days after challenge. The eighth challenged calf scoured severely and was killed in extremis 7 days after challenge. Four unvaccinated control calves infected orally with ST4/74 28 days after birth all scoured severely and were euthanased for humane reasons 5-7 days later. This study suggests that **vaccination** with an attenuated strain of S. typhimurium harbouring **mutations** in two aro genes is a safe and effective way of protecting young calves against experimental S. typhimurium infection.

L18 ANSWER 54 OF 59 MEDLINE DUPLICATE 30
ACCESSION NUMBER: 91181307 MEDLINE
DOCUMENT NUMBER: 91181307 PubMed ID: 2008803
TITLE: Construction of aromatic dependent Shigella flexneri 2a live **vaccine** candidate strains: deletion **mutations** in the aroA and the **aroD** genes.
AUTHOR: Verma N K; Lindberg A A
CORPORATE SOURCE: Karolinska Institute, Dept. Clinical Bacteriology, Huddinge Hospital, Sweden.
SOURCE: VACCINE, (1991 Jan) 9 (1) 6-9.
Journal code: X6O; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910519
Last Updated on STN: 19910519
Entered Medline: 19910501

AB Live Shigella flexneri 2a **vaccine** candidate strains with deletion **mutations** in the aro genes were constructed. Tn10-generated auxotrophic **mutations** were transduced from Escherichia coli to S. flexneri 2a with bacteriophage P1CmCts. The tetracycline-sensitive derivatives of Tn10 **mutants** obtained were selected on Bochner's medium and checked by DNA-DNA hybridization using aroA and **aroD** gene specific probes. The **vaccine** candidate strains were tested to assess the efficacy of protection in guinea-pig conjunctival epithelia (Sereny test). The strains did not cause keratoconjunctivitis and exhibited significant protection in the challenge experiments. A candidate **vaccine** strain (delta **aroD**) showed 100% protection against 10(7) c.f.u. of wild type strain in the **immunized** guinea-pigs.

L18 ANSWER 55 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1990-363446 [49] WPIDS
DOC. NO. CPI: C1990-157936
TITLE: Attenuated **bacteria** for use as live **vaccines** - have gene mutated, which regulates one or more genes concerned with outer membrane proteins esp. porin.
DERWENT CLASS: B04 C03 D16
INVENTOR(S): CHATFIELD, S N; DORMAN, C J; DOUGAN, G; HIGGINS, C F; CHARFIELD, S N
PATENT ASSIGNEE(S): (LIST-N) LISTER INST PREVENTIVE MEDICINE; (ROYA-N) ROYAL SOC; (UYDU-N) UNIV DUNDEE; (WELL) WELLCOME FOUND LTD; (LIST-N) LISTER INST PREVENTIVE M; (GLAX) GLAXO WELLCOME INC
COUNTRY COUNT: 16
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 400958	A	19901205	(199049)*		9
R: AT BE CH DE ES FR GB GR IT LI LU NL SE					
JP 03117481	A	19910520	(199126)		
EP 400958	B1	19950913	(199541)	EN	12
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE					
DE 69022290	E	19951019	(199547)		
ES 2077028	T3	19951116	(199551)		
US 5527529	A	19960618	(199630)		7
US 5851519	A	19981222	(199907)		
JP 3024982	B2	20000327	(200020)		7

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 400958	A	EP 1990-305819	19900529
JP 03117481	A	JP 1990-139566	19900529
EP 400958	B1	EP 1990-305819	19900529

DE 69022290	E		DE 1990-622290	19900529
			EP 1990-305819	19900529
ES 2077028	T3		EP 1990-305819	19900529
US 5527529	A	Cont of	US 1990-528972	19900529
		Cont of	US 1992-827584	19920127
			US 1995-419741	19950410
US 5851519	A	Cont of	US 1990-528972	19900529
		Div ex	US 1992-827584	19920127
			US 1995-419618	19950410
JP 3024982	B2		JP 1990-139566	19900529

FILING DETAILS:

PATENT NO	KIND		PATENT NO
DE 69022290	E	Based on	EP 400958
ES 2077028	T3	Based on	EP 400958
JP 3024982	B2	Previous Publ.	JP 03117481

PRIORITY APPLN. INFO: GB 1989-12330 19890530
AN 1990-363446 [49] WPIDS
AB EP 400958 A UPAB: 19970417
An attenuated **bacteria**, with a **mutation** in a gene concerned with regulating one or more additional genes, is new. The genes regulated encode an outer membrane protein and are porin genes. The regulating gene is Omp. R. The **bacteria** is gram negative and selected from Salmonella, **Bordetella**, Viloris, **Haemophilus** and Escherichia genera, pref. it is from S. typhi an A-, omp-, S. typhimium omp-, or aroA-, omp R- or S. dublin omp R- or aroA, ompR-. Opt. a second gene is also **mutated**, which encodes an enzyme involved in an essential auxotrophic pathway. This gene is pref. anoA, aroC, or **aroD**

USE/ADVANTAGE - **Bacteria** attenuated in such a way that can be used as live **vaccines** in human and animal medicine. It can be used in a prophylactic treatment of a **bacterial** infection, in an effective dose which depends on various clinical factors. For S.typhi a dosage of 109-1011 organisms/dose is used for a 70 kg human patient. @(9pp Dwg.No.0/1)@

ABEQ EP 400958 B UPAB: 19951019
A **vaccine** formulation comprising a **bacterium** attenuated by a non-revering mutation in the ompR gene in admixture with a pharmaceutically acceptable excipient.
Dwg.0/0

ABEQ US 5527529 A UPAB: 19960731
A pharmaceutical composition for oral administration to a subject for inducing immunity to a pathogenic Salmonella **bacterium**, which composition comprises a pharmaceutically acceptable excipient and an attenuation form of said Salmonella **bacterium**, the attenuation being attributable to a non-reverting mutation in the ompR gene of said Salmonella **bacterium**.
Dwg.0/1

L18 ANSWER 56 OF 59 MEDLINE DUPLICATE 31
ACCESSION NUMBER: 91326956 MEDLINE
DOCUMENT NUMBER: 91326956 PubMed ID: 1714093
TITLE: Aromatic-dependent Salmonella as live **vaccine**

presenters of foreign epitopes as inserts in flagellin.

AUTHOR: Stocker B A
CORPORATE SOURCE: Department of Microbiology and Immunology, Stanford University School of Medicine, CA 94305-5402.

CONTRACT NUMBER: AI-18872 (NIAID)
AI-27722 (NIAID)
SOURCE: RESEARCH IN MICROBIOLOGY, (1990 Sep-Oct) 141 (7-8) 787-96.
Journal code: R6F; 8907468. ISSN: 0923-2508.
PUB. COUNTRY: France
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199109
ENTRY DATE: Entered STN: 19910929
Last Updated on STN: 19960129
Entered Medline: 19910909

AB Synthetic oligonucleotides specifying amino acid sequences identified as epitopes of various foreign antigens (cholera toxin subunit B, hepatitis B surface protein and others) have been inserted at an EcoRV-EcoRV deletion site in a cloned Salmonella flagellin gene; the resulting plasmids, when placed in flagellin-negative Escherichia coli or Salmonella sp. strains, caused production of flagellin expressing the epitope. If the chimeric flagellin allowed formation of flagella, the epitope was exposed at the surface of the flagellar filaments. A delta aroA flagellin-negative S. dublin live **vaccine** strain given plasmids carrying various chimeric flagellin genes was administered to mice, etc. Serum antibody specific for the foreign epitope was in all cases evoked by parenteral administration; oral route administration was effective in the case of two epitopes of hepatitis B surface protein but not effective for several other epitopes. Several i.p. inocula of the live **vaccine** strain with an insert corresponding to the 15 N-terminal amino acids of the M protein of Streptococcus pyogenes type 5 evoked M-specific antibody with opsonic activity, and the mice were (incompletely) protected against a lethal challenge of S. pyogenes type 5. The non-virulence of Salmonella sp. strains with complete blocks in the aromatic biosynthesis pathway, even for animals with genetically determined or other defects in host defences, can be completely accounted for by their requirement for p-aminobenzoic acid, since non-leaky pabB **mutations** caused similar attenuation. Two transposon insertions at **aroE** caused little or no attenuation, presumably because they did not result in complete block of the relevant step in biosynthesis. The limited growth of delta aroA strains in mouse tissues parallels that which precedes the bacteriostasis caused by addition of a sulphonamide to a growing broth culture of a sulphonamide-sensitive strain; the final cessation of growth in each case presumably results from inability to initiate new protein chains with a formyl-methionine unit when the original folic acid content of the **bacteria** has been diluted out by residual growth.

L18 ANSWER 57 OF 59 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1990:89320 BIOSIS
DOCUMENT NUMBER: BA89:48671
TITLE: TRANSPOSON-GENERATED TN10 INSERTION MUTATIONS AT THE

ARO GENES OF ESCHERICHIA-COLI K-12.
AUTHOR(S): COBOS A; FERNANDEZ M F; HERNANDEZ P E; SANZ B
CORPORATE SOURCE: DPTO. HIG. TECNOL. ALIMENTOS, FAC. VET., UNIV. COMPLUTENSE, 28040 MADRID, SPAIN.
SOURCE: CURR MICROBIOL, (1990) 20 (1), 13-18.
CODEN: CUMIDD. ISSN: 0343-8651.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB We have obtained a set of Escherichia coli K-12 derivatives with transposon-generated Tn10 insertion **mutations** at the aro genes of their aromatic biosynthetic pathway. Bacteriophage .lambda.NK561 (Tn10) has been used for transposon **mutagenesis** of E. coli, strain BW545. Tetracycline (Tc)-resistant derivatives were screened by their Aro- phenotype by growth on a minimal medium with adequate requirements. Six aro **mutant** types were mapped; two strains were aroA, two **aroD**, one aroB or **aroE**, and one aroC. A selective medium and a D-cycloserine enrichment in the presence of tetracycline were used to select for Aro-, Tc-sensitive derivatives. The reversion index to aromatic-independent colonies of some derivatives was less than 2 .times. 10-11 per **bacterium** per generation. P1 transduction experiments transferred an aroA::Tn10 insertion from E. coli BW545 to an enterotoxigenic E. coli strain from porcine origin. Derivatives of this strain being aro, Tc-sensitive and not reverting to aro+ at a detectable frequency, and many others transduced at will, may prove their usefulness as live **vaccines**.

L18 ANSWER 58 OF 59 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1989-309381 [42] WPIDS
CROSS REFERENCE: 1985-289426 [46]; 1986-155753 [24]; 1989-206100 [28]
DOC. NO. CPI: C1989-136955
TITLE: Non-reverting live Shigella **vaccines** - having a requirement for an essential metabolite which is not available in a mammalian host.
DERWENT CLASS: B04 D16
INVENTOR(S): STOCKER, B A D; STOCKER, B A
PATENT ASSIGNEE(S): (STRD) UNIV LELAND STANFORD JUNIOR
COUNTRY COUNT: 20
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 8909063	A	19891005	(198942)*	EN	60
RW: AT BE CH DE FR GB IT LI LU NL SE					
W: AU DK FI HU JP NO					
PT 90075	A	19891110	(198950)		
FI 8904446	A	19890922	(199002)		
NO 8904495	A	19891227	(199006)		
AU 8935520	A	19891016	(199008)		
DK 8905565	A	19891108	(199015)		
EP 368966	A	19900523	(199021)		
R: AT BE CH DE FR GB IT LI LU NL SE					
JP 02503868	W	19901115	(199101)		
HU 53810	T	19901228	(199107)		
US 5077044	A	19911231	(199204)		17
US 5210035	A	19930511	(199320)		17

AU 640344	B	19930826 (199341)	
EP 368966	A4	19910109 (199514)	
CA 1335661	C	19950523 (199528)	
EP 368966	B1	19960508 (199623) EN	12
R: AT BE CH DE FR GB IT LI LU NL SE			
DE 68926431	E	19960613 (199629)	
US 5643771	A	19970701 (199732)	17
FI 100945	B1	19980331 (199819)	

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 8909063	A		WO 1989-US847	19890302
EP 368966	A		EP 1989-905556	19890302
US 5077044	A		US 1988-170727	19880321
US 5210035	A	CIP of	US 1980-151002	19800519
		CIP of	US 1982-415291	19820907
		CIP of	US 1984-675381	19841127
		CIP of	US 1985-798052	19851114
		Cont of	US 1988-170727	19880321
			US 1991-745876	19910816
AU 640344	B		AU 1989-35520	19890302
EP 368966	A4		EP 1989-905556	
CA 1335661	C		CA 1989-593620	19890314
EP 368966	B1		EP 1989-905556	19890302
			WO 1989-US847	19890302
DE 68926431	E		DE 1989-626431	19890302
			EP 1989-905556	19890302
			WO 1989-US847	19890302
US 5643771	A	CIP of	US 1980-151002	19800519
		CIP of	US 1982-415291	19820907
		CIP of	US 1984-675381	19841127
		CIP of	US 1985-798052	19851114
		Cont of	US 1991-745876	19910816
		Cont of	US 1993-16579	19930210
			US 1994-293407	19940819
FI 100945	B1		WO 1989-US847	19890302
			FI 1989-4446	19890920

FILING DETAILS:

PATENT NO	KIND		PATENT NO
US 5210035	A	CIP of	US 4550081
		CIP of	US 4735801
		CIP of	US 4837151
		Cont of	US 5077044
AU 640344	B	Previous Publ.	AU 8935520
		Based on	WO 8909063
EP 368966	B1	Based on	WO 8909063
DE 68926431	E	Based on	EP 368966
		Based on	WO 8909063
US 5643771	A	CIP of	US 4550081
		CIP of	US 4735801
		CIP of	US 4837151
		Cont of	US 5210035
FI 100945	B1	Previous Publ.	FI 8904446

Searcher : Shears 308-4994

PRIORITY APPLN. INFO: US 1988-170727 19880321; US 1980-151002 19800519; US 1982-415291 19820907; US 1984-675381 19841127; US 1985-798052 19851114; US 1991-745876 19910816; US 1993-16579 19930210; US 1994-293407 19940819

AN 1989-309381 [42] WPIDS

CR 1985-289426 [46]; 1986-155753 [24]; 1989-206100 [28]

AB WO 8909063 A UPAB: 19960227

The following are claimed as new (A) a live Shigella strain having a requirement for at least one essential metabolite which is not available in a mammalian host, the requirement being as a result of a non-reverting **mutation**; (B) a live Shigella strain having the following properties: non-reverting **aroD-**; Sereny negative; Congo red positive; serotype Y; sensitivity to antibiotics, and ability to grow on chemically defined media; (C) a **vaccine** strian of Shigella flexneri least one essential metabolite which is not available in a mammalian host, the requirement being as a result of a non-reverting deletion or deletion-inversion, grow on chemically defined media, are sensitive to antibiotics, are Sereny-negative and comprise the invasiveness plasmid; (D) Shigella flexneri strain SFL 114, ATCC 53755.

USE/ADVANTAGE - The auxotrophic **vaccine** strains provided have non-reverting blocks in a biosynthetic pathway which ensure that though the strains live in a host organism they are unable to be proliferated. The mutated organisms retain the same antigenic characteristics as the unmutated, virulent organisms, thus inducing a protective immune response.

Dwg.0/0

Dwg.0/0

ABEQ US 5077044 A UPAB: 19930923

Live Shigella strains, e.g. Shigella flexneri, serotypes 1a, 1b, 2a, 2b, 3a, 4a, 4b and 5 (obtd. by lysogenisation of a serotype Y with one or more bacteriophages) are new strains which require one or more essential metabolites not normally present in a mammalian host. These strains do not revert to aromatic D-(-)-aminoacids, are Sereney negative but Congo red positive, and are sensitive to numerous antibiotics.

USE - The prods. are components for **vaccines** against dysentery, but other microorganism strains can be modified in a similar manner to provide a wide range of **vaccines**. @

ABEQ US 5210035 A UPAB: 19931113

Prepn. of a live non-virulent **vaccine** from a virulent pathogenic microorganism, comprises subjecting a strain of microorganism to mutation, giving a mutated microorganism having at least two non-reverting mutations. Mutations involve at least 5 nucleotides each and result in a block in at least one biosynthetic pathway which renders the organism auxotrophic with a metabolite normally unavailable in a host. Mutations comprise at least one of a deletion, insertion or inversion. Non-reverting mutated microorganism is then selected for.

Also claimed is a **vaccine** comprising the mutant.

USE/ADVANTAGE - As a **vaccine** against Salmonella and Shigella. Does not revert to virulence.

Dwg.0/0

ABEQ EP 368966 B UPAB: 19960610

Shigella flexneri strain SFL114, ATCC Accession No. 53755, or mutants or derivatives thereof.

Dwg.0/0
ABEQ US 5643771 A UPAB: 19970806
Preparation of a live non-virulent **vaccine** from a virulent pathogenic **bacterial** microorganism, the **vaccine** being substantially incapable of reverting to virulence in a vertebrate host susceptible to the microorganism, h comprises:

(a) subjecting a virulent strain of said microorganism to mutating conditions resulting in a mutated microorganism having at least two non-reverting mutations involving at least five nucleotides each and resulting in a block in at least one biosynthetic pathway which renders the organism auxotrophic with a requirement for a metabolite normally unavailable in a host susceptible to said microorganism, the mutations comprising at least one of deletion, insertion or inversion;

(b) selecting for non-reverting mutated microorganism;

(c) isolating non-reverting mutated microorganism to provide a living **vaccine**;

(d) introducing an expression cassette containing a DNA sequence encoding an antigen foreign to said pathogenic microorganism, under regulatory control of regulatory regions recognized by said pathogenic microorganism, into said pathogenic microorganism or mutant microorganism to produce a transformed host cell;

(e) growing said transformed host cell; and

(f) identifying and isolating transformed host cells expressing said antigen;

wherein (d), (e) and (f) may be carried out before or after any one of (a) through (c), resulting in a culture of auxotrophic, non-reverting, non-virulent mutant microorganism capable of expressing an antigen foreign to said microorganism.
Dwg.0/0

L18 ANSWER 59 OF 59 MEDLINE
ACCESSION NUMBER: 89218937 MEDLINE
DOCUMENT NUMBER: 89218937 PubMed ID: 2523513
TITLE: Bacteriophage P22 as a vehicle for transducing cosmid gene banks between smooth strains of Salmonella typhimurium: use in identifying a role for aroD in attenuating virulent Salmonella strains.
AUTHOR: Miller I A; Chatfield S; Dougan G; Desilva L; Joysey H S; Hormaeche C
CORPORATE SOURCE: Department of Pathology, University of Cambridge, UK.
SOURCE: MOLECULAR AND GENERAL GENETICS, (1989 Jan) 215 (2) 312-6.
Journal code: NGP; 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198906
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 19900306
Entered Medline: 19890608

AB A cosmid gene bank of the virulent Salmonella typhimurium C5 was constructed in Escherichia coli K12. The bank was repackaged into bacteriophage heads and transduced into the semi-rough S. typhimurium strain AS68 which expresses the LamB lambda receptor protein. Approximately 6000 ampicillin-resistant transductants were

pooled and used as host for the propagation of bacteriophage P22. The P22 lysate was able to transduce cosmid recombinants to smooth strains of S. typhimurium and individual transductants were selected which complemented various S. typhimurium auxotrophic **mutations**. A stable **mutation** was introduced into the **aroD** gene of S. typhimurium C5. The resulting **aroD- mutant**, named CU038, was highly attenuated compared with the wild-type parent strain and BALB/c mice **immunised** orally with CU038 were well protected against challenge with the virulent C5 parental strain. Using the cosmid bank repackaged into bacteriophage P22 heads it was possible to isolate cosmid recombinants that could complement the **aroD mutation** of CU038 either by in vitro selection using minimal medium or in vivo selection for restoration of virulence in BALB/c mice. Repackaged P22 cosmid banks could provide a simple system for selecting in vivo for Salmonella virulence determinants. A Salmonella typhi strain harbouring **mutations** in aroA and **aroD** was constructed for potential use as a live oral typhoid **vaccine** in humans.

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 12:54:47 ON 06 FEB 2002)

L19	478	S	CHATFIELD S?/AU
L20	1391	S	DOUGAN G?/AU
L21	66	S	SYDENHAM M?/AU
L22	8	S	L19 AND L20 AND L21
L23	265	S	L19 AND (L20 OR L21)
L24	8	S	L20 AND L21
L25	1662	S	L19 OR L20 OR L21
L26	92	S	(L23 OR L25) AND L2
L27	56	S	(L23 OR L25) AND L15
L28	58	S	L22 OR L24 OR L27
L29	28	DUP REM	L28 (30 DUPLICATES REMOVED)

- Author(s)

L29 ANSWER 1 OF 28 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2001021219 MEDLINE
DOCUMENT NUMBER: 20448972 PubMed ID: 10992518
TITLE: Comparison of abilities of Salmonella enterica serovar typhimurium aroA **aroD** and aroA **htrA mutants** to act as live vectors.
AUTHOR: Roberts M; **Chatfield S**; Pickard D; Li J; Bacon A
CORPORATE SOURCE: Department of Veterinary Pathology, Glasgow University Veterinary School, Glasgow G61 1QH, United Kingdom.. M.Roberts@vet.gla.ac.uk
SOURCE: INFECTION AND IMMUNITY, (2000 Oct) 68 (10) 6041-3. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001103

AB We compared the ability of Salmonella enterica serovar Typhimurium SL1344 aroA **aroD** (BRD509) and aroA **htrA** (BRD807)

mutants to act as live vectors for delivery of fragment C of tetanus toxin (FrgC). FrgC was expressed in these strains from either pTETnir15 or pTEThtrA1. BRD509FrgC(+) strains elicited approximately 2-log-higher serum anti-FrgC antibody titers than BRD807FrgC(+) strains. All mice immunized with BRD807pTEThtrA1, BRD509pTEThtrA1, and BRD509pTETnir15 (but not BRD807pTETnir15) were protected against tetanus.

L29 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 2000:831674 CAPLUS
DOCUMENT NUMBER: 134:70048
TITLE: Susceptibility of calves to challenge with Salmonella typhimurium 4/74 and derivatives harbouring **mutations** in **htrA** or purE
AUTHOR(S): Villarreal-Ramos, Bernardo; Manser, Jacquie M.; Collins, Robert A.; Chance, Victoria; Eckersall, P. David; Jones, Phillip W.; **Dougan, Gordon**
CORPORATE SOURCE: Institute for Animal Health, Compton, RG20 7NN, UK
SOURCE: Microbiology (Reading, U. K.) (2000), 146(11), 2775-2783
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Salmonella typhimurium 4/74 is highly virulent for cattle after oral challenge, causing severe diarrhea, which is sometimes assocd. with systemic spread of the micro-organism. Although susceptible to oral challenge, groups of cattle were found to be relatively resistant to s.c. challenge with this strain. The virulence of S. typhimurium 4/74 harbouring **mutations** in **htrA** and purE was also assessed in cattle. Although S. typhimurium 4/74 **htrA** and purE are attenuated following oral challenge in mice, cattle were highly susceptible to oral challenge with these **mutants**. As with the parent S. typhimurium 4/74 strain, cattle exhibited greater susceptibility to oral compared to s.c. challenge with S. typhimurium **htrA** and purE **mutants**. Following s.c. challenge with sublethal levels of S. typhimurium 4/74, calves produced significant levels of antibodies to S. typhimurium sol. ext. No correlation was detected between interferon gamma levels in sera and susceptibility to infection by any route. The concns. of the acute-phase-assocd. protein haptoglobin were increased in the sera of five of six cattle inoculated s.c., although increases in concn. were smaller in cattle inoculated orally.
REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 3 OF 28 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2000087308 MEDLINE
DOCUMENT NUMBER: 20087308 PubMed ID: 10618549
TITLE: Cattle immune responses to tetanus toxoid elicited by recombinant S. typhimurium vaccines or tetanus toxoid in alum or Freund's adjuvant.
AUTHOR: Villarreal-Ramos B; Manser J M; Collins R A; **Dougan G**; Howard C J

CORPORATE SOURCE: Institute for Animal Health, Compton, Newbury, UK..
bernardo.villareal@bbsrc.ac.uk
SOURCE: VACCINE, (2000 Feb 14) 18 (15) 1515-21.
Journal code: X6O; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000330
Last Updated on STN: 20000330
Entered Medline: 20000321

AB Cattle were immunised orally, nasally or subcutaneously with either S. typhimurium 4/74 aroA(-) **aroD**(-) or S. typhimurium 4/74 **htrA**-based live vaccines expressing Fragment C (TetC) of tetanus toxin from plasmid pTetnir15. Oral inoculation with S. typhimurium 4/74 aroA(-) **aroD**(-)- (pTetnir15) elicited mucosal anti-TetC IgA but no measurable systemic humoral responses to TetC. Subcutaneous inoculation with the same strain elicited both mucosal IgA and systemic anti-TetC IgG1 responses. Nasal inoculation did not elicit any detectable anti-TetC responses. Oral delivery of S. typhimurium **htrA**(-) proved fatal in inoculated animals. None of the animals inoculated with either **mutant** S. typhimurium developed detectable T cell proliferative responses to the guest antigen. Cattle were also inoculated with tetanus toxoid adsorbed in alum or emulsified in Freund's complete adjuvant. Animals inoculated subcutaneously with Ttox emulsified in FCA developed systemic IgG1 and IgG2 antibody, while animals inoculated with Ttox adsorbed in alum developed systemic IgG1 but little IgG2 to Ttox. Both of these groups of animals developed measurable TetC-specific proliferative T cell responses that were associated with the production of IFNgamma.

L29 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
ACCESSION NUMBER: 2000:146770 CAPLUS
DOCUMENT NUMBER: 132:292412
TITLE: Salmonella enterica serovar typhimurium surA mutants are attenuated and effective live oral vaccines
AUTHOR(S): **Sydenham, Mark**; Douce, Gillian; Bowe, Frances; Ahmed, Saddif; **Chatfield, Steve**; **Dougan, Gordon**
CORPORATE SOURCE: Medeva Vaccine Development Group, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK
SOURCE: Infect. Immun. (2000), 68(3), 1109-1115
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A previously described attenuated TnphoA mutant (BRD441) of Salmonella enterica serovar Typhimurium C5 was characterized, and the transposon was shown to be inserted in surA, a gene which encodes a peptidylprolyl-cis,trans-isomerase. A defined surA deletion mutation was introduced into S. enterica serovar Typhimurium C5 and the mutant strain, named S. enterica serovar Typhimurium BRD1115, was extensively characterized both in vitro and in vivo. S. enterica serovar Typhimurium BRD1115 was defective in

the ability to adhere to and invade eukaryotic cells. Furthermore, S. enterica serovar Typhimurium BRD1115 was attenuated by at least 3 log units when administered orally or i.v. to BALB/c mice. Complementation of the mutation with a plasmid carrying the intact surA gene almost completely restored the virulence of BRD1115. In addn., S. enterica serovar Typhimurium BRD1115 demonstrated potential as a vaccine candidate, since mice immunized with BRD1115 were protected against subsequent challenge with S. enterica serovar Typhimurium C5. S. enterica serovar Typhimurium BRD1115 also showed potential as a vehicle for the effective delivery of heterologous antigens, such as the nontoxic, protective fragment C domain of tetanus toxin, to the murine immune system.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 5
ACCESSION NUMBER: 2000:189522 CAPLUS
DOCUMENT NUMBER: 133:16055
TITLE: Kinetics of the mucosal antibody secreting cell response and evidence of specific lymphocyte migration to the lung after oral immunisation with attenuated S. enterica var. typhimurium
AUTHOR(S): Allen, J. S.; **Dougan, G.**; Strugnell, R. A.
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Melbourne, Parkville, Australia
SOURCE: FEMS Immunol. Med. Microbiol. (2000), 27(4), 275-281
CODEN: FIMIEV; ISSN: 0928-8244
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The kinetic of mucosal secretory responses elicited by the vaccine vector Salmonella enterica var. typhimurium (S. typhimurium) was examd. by enzyme linked immunospot (ELISPOT) and compared with serum responses. Mice immunized orally with BRD509, the aroA, **aroD mutant** of virulent S. typhimurium SL1344 expressing the C Fragment of tetanus toxin (TT), simultaneously developed an IgA antibody secreting cells (ASC) response in the gastrointestinal lamina propria, the spleen and the lung, against both S. typhimurium lipopolysaccharide (LPS) and TT. The magnitude of the ASC response was greatest in the gut, was boosted by a secondary immunization at day 25, and the kinetic of the response did not correlate with the appearance of serum antibodies. This study suggests that S. typhimurium can engage the common mucosal immune system to effect mucosal secretory responses at distal sites, however, the magnitude of the responses is both greatest in the gut and antigen-specific. The ASC origin of the serum antibodies specific for S. typhimurium and antigens expressed by the **bacterium** is yet to be elucidated.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 6 OF 28 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6
ACCESSION NUMBER: 1999:388086 CAPLUS
DOCUMENT NUMBER: 131:43576

TITLE: Vaccines containing attenuated **bacteria**
INVENTOR(S): **Chatfield, Steven Neville; Sydenham, Mark; Dougan, Gordon**
PATENT ASSIGNEE(S): Medeva Europe Limited, UK
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9929342	A1	19990617	WO 1998-GB3680	19981210
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9914960	A1	19990628	AU 1999-14960	19981210
AU 739191	B2	20011004		
EP 1037664	A1	20000927	EP 1998-959023	19981210
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2001525375	T2	20011211	JP 2000-524011	19981210

PRIORITY APPLN. INFO.: GB 1997-26233 A 19971211
WO 1998-GB3680 W 19981210

AB The invention relates to a vaccine comprising a **bacterium** attenuated by a non-reverting **mutation** in a gene, e.g. **surA** gene and gene for parvulin (peptidyl-prolyl cis-trans isomerase), encoding a protein which promotes folding of extracytoplasmic proteins. Such mutations were initially identified as being useful in vaccines from a bank of randomly inserted, transposon mutants in which attenuation was detd. as a redn. in virulence of the organism in the mouse model of infection. Site directed mutation of the gene results in a strain which shows at least 4 logs of attenuation when delivered both orally and i.v. Animals vaccinated with such a strain are protected against subsequent challenge with the parent wild type strain. Finally, heterologous antigens such as the non-toxic and protective, binding domain from tetanus toxin, fragment C, can be delivered via the mucosal immune system using such strains of **bacteria**. This results in the induction of a fully protective immune response to subsequent challenge with native tetanus toxin.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 7 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:626318 CAPLUS
DOCUMENT NUMBER: 131:253344
TITLE: **Bacteria** attenuated by a non-reverting mutation in each of the aroC, ompF and ompC genes, useful as vaccines

INVENTOR(S): **Chatfield, Steven Neville**
PATENT ASSIGNEE(S): Peptide Therapeutics Limited, UK
SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9949026	A1	19990930	WO 1999-GB935	19990325
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9930458	A1	19991018	AU 1999-30458	19990325
EP 1066376	A1	20010110	EP 1999-911949	19990325
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
NO 2000004781	A	20001108	NO 2000-4781	20000925

PRIORITY APPLN. INFO.: GB 1998-6449 A 19980325
WO 1999-GB935 W 19990325

AB The invention provides a **bacterium** attenuated by a non-reverting mutation in each of the aroC gene, the ompF gene and the ompC gene. The **bacterium** is useful as a vaccine. The **bacterium** may, for example, be an attenuated strain of E. coli useful in vaccination against diarrhea. Thus, the design of deletions and construction of plasmids is described for removal of the entire open reading frame of target aroC, ompC, and ompF genes from the E1392/75/2A strain of enterotoxigenic E. coli. The attenuated vaccine strain (.DELTA.aroc/.DELTA.ompc/.DELTA.ompF) is well tolerated in healthy adult volunteers and colonizes the intestine in a manner consistent with its utility as an oral vaccine to protect against travelers diarrhea. It has also been demonstrated to elicit a specific mucosal immune response.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 8 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:290239 BIOSIS
DOCUMENT NUMBER: PREV200000290239
TITLE: Vaccines containing **bacteria** attenuated by **mutation** of the **htrA** gene.
AUTHOR(S): **Dougan, Gordan (1)**; Charles, Ian George; Hormaeche, Carlos Estenio; Johnson, Kevin Stuart; **Chatfield, Steven Neville**
CORPORATE SOURCE: (1) Beckenham UK
ASSIGNEE: Glaxo Wellcome Inc., Research Triangle Park, NC, USA
PATENT INFORMATION: US 5980907 November 09, 1999
SOURCE: Official Gazette of the United States Patent and

Trademark Office Patents, (Nov. 9, 1999) Vol. 1228, No. 2, pp. No pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
AB Attenuated microorganism for use in immunoprophylaxis in which the attenuation is brought about by the presence of a mutation in the DNA sequence of the microorganism which encodes, or which regulates the expression of DNA encoding a protein that is produced in response to environmental stress, the microorganism optionally being capable of expressing DNA encoding a heterologous antigen.

L29 ANSWER 9 OF 28 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1999115546 MEDLINE
DOCUMENT NUMBER: 99115546 PubMed ID: 9916080
TITLE: Characterization of candidate live oral Salmonella typhi vaccine strains harboring defined **mutations** in aroA, aroC, and **htrA**.
AUTHOR: Lowe D C; Savidge T C; Pickard D; Eckmann L; Kagnoff M F; **Dougan G; Chatfield S N**
CORPORATE SOURCE: Department of Cellular Physiology, The Babraham Institute, Babraham, Cambridge CB2 4AT, Imperial College of Science, Technology and Medicine, London SW7 2AY, United Kingdom.
SOURCE: INFECTION AND IMMUNITY, (1999 Feb) 67 (2) 700-7. Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 20000303
Entered Medline: 19990309
AB The properties of two candidate Salmonella typhi-based live oral typhoid vaccine strains, BRD691 (S. typhi Ty2 harboring **mutations** in aroA and aroC) and BRD1116 (S. typhi Ty2 harboring **mutations** in aroA, aroC, and **htrA**), were compared in a number of in vitro and in vivo assays. BRD1116 exhibited an increased susceptibility to oxidative stress compared with BRD691, but both strains were equally resistant to heat shock. Both strains showed a similar ability to invade Caco-2 and HT-29 epithelial cells and U937 macrophage-like cells, but BRD1116 was less efficient at surviving in epithelial cells than BRD691. BRD1116 and BRD691 were equally susceptible to intracellular killing within U937 cells. Similar findings were demonstrated in vivo, with BRD1116 being less able to survive and translocate to secondary sites of infection when inoculated into the lumen of human intestinal xenografts in SCID mice. However, translocation of BRD1116 to spleens and livers in SCID mice occurred as efficiently as that of BRD691 when inoculated intraperitonally. The ability of BRD1116 to increase the secretion of interleukin-8 following infection of HT-29 epithelial cells was comparable to that of BRD691. Therefore, loss of the **HtrA** protease in S. typhi does not seem to alter its ability to invade epithelial cells or macrophages or to induce proinflammatory cytokines such as IL-8 but significantly reduces intracellular survival in human intestinal epithelial cells in vitro and in vivo.

L29 ANSWER 10 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:124154 BIOSIS
DOCUMENT NUMBER: PREV200200124154
TITLE: Vaccines containing a salmonella **bacteria** attenuated by **mutation** of the **htrA** gene.
AUTHOR(S): **Dougan, G.**; Charles, I. G.; Hormaeche, C. E.; Johnson, K. S.; **Chatfield, S. N.**
CORPORATE SOURCE: Beckenham, United Kingdom
ASSIGNEE: GLAXO WELLCOME INC.
PATENT INFORMATION: US 5804194 Sept. 8, 1998
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Sept. 8, 1998) Vol. 1214, No. 2, pp. 1692.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

L29 ANSWER 11 OF 28 MEDLINE
ACCESSION NUMBER: 1998230472 MEDLINE
DOCUMENT NUMBER: 98230472 PubMed ID: 9570545
TITLE: Protective effect on Leishmania major infection of migration inhibitory factor, TNF-alpha, and IFN-gamma administered orally via attenuated Salmonella typhimurium.
AUTHOR: Xu D; McSorley S J; Tetley L; **Chatfield S**; **Dougan G**; Chan W L; Satoskar A; David J R; Liew F Y
CORPORATE SOURCE: Department of Immunology, University of Glasgow, United Kingdom.
SOURCE: JOURNAL OF IMMUNOLOGY, (1998 Feb 1) 160 (3) 1285-9.
Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980520
Last Updated on STN: 19990129
Entered Medline: 19980514

AB The genes encoding murine macrophage migration inhibitory factor (MIF), IL-2, IFN-gamma or TNF-alpha were cloned individually into an expression plasmid under the control of the inducible promoter **nirB** and transfected into the aroA- **aroD**- deletion **mutant** strain of Salmonella typhimurium (BRD509). These S. typhimurium derivatives (henceforward called constructs and termed GIDMIF, GIDIL2, GIDIFN and GIDTNF) expressed their respective cytokines in vitro under anaerobic conditions and stably colonized BALB/c mice up to 14 days after oral administration. The highly susceptible BALB/c mice that had received the constructs orally and that had been subsequently infected via the footpad with Leishmania major, developed significantly reduced disease compared with control mice administered the untransfected Salmonella strain (BRD509). Importantly, a combination of GIDMIF, GIDIFN, and GIDTNF administered orally after L. major infection was able to significantly limit lesion development and reduced parasite loads by up to three orders of magnitude. Spleen and lymph node cells of mice

administered this combination expressed markedly higher levels of inducible nitric oxide synthase (iNOS) compared with those from mice receiving an equivalent dose of the control strain of Salmonella (BRD509). These data therefore demonstrate the feasibility of therapeutic treatment in an infectious disease model using cytokines delivered by attenuated Salmonella. The protective effect observed correlates with the induction of inducible nitric oxide synthase in vivo.

L29 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:358711 CAPLUS
DOCUMENT NUMBER: 127:120395
TITLE: Attenuated Salmonella typhi and Shigella as live oral vaccines and as live vectors
AUTHOR(S): Levine, M. M.; Galen, J.; Barry, E.; Noriega, F.; Tacket, C.; Sztein, M.; **Chatfield, S.**; **Dougan, G.**; Losonsky, G.; Kotloff, K.
CORPORATE SOURCE: School Medicine, Univ. Maryland, Baltimore, MD, 21201, USA
SOURCE: Behring Inst. Mitt. (1997), 98(New Approaches to Bacterial Vaccine Development), 120-123
CODEN: BHIMA2; ISSN: 0301-0457
PUBLISHER: Medizinische Verlagsgesellschaft mbH
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review is given with 26 refs. including the authors own works on new generations of attenuated Salmonella typhi and Shigella strains with precise, defined **mutations** for use as live oral vaccines and on the live vectors CVD 908 and CVD 908-**htrA**.

L29 ANSWER 13 OF 28 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 8
ACCESSION NUMBER: 1996:327978 CAPLUS
DOCUMENT NUMBER: 125:27332
TITLE: Construction and characterization of a **Yersinia** enterocolitica O:8 high-temperature requirement (**htrA**) isogenic **mutant**
AUTHOR(S): Li, shu-Rui; Dorrell, Nick; Everest, Paul H.; **Dougan, Gordon**; Wren, Brendan W.
CORPORATE SOURCE: Department Medical Microbiology, Imperial College Science, London, UK
SOURCE: Infect. Immun. (1996), 64(6), 2088-2094
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The high-temp. requirement (HtrA) family of stress response proteins are induced by different environmental stress conditions in a variety of **bacteria** and have been shown to contribute to the pathogenicity of some of these species. In this study, the htrA gene from **Yersinia** enterocolitica O:8 was amplified, cloned, and sequenced. Anal. of the deduced amino acid sequence predicted that the putative HtrA homolog contains a serine protease active site and a catalytic triad characteristic of trypsin-like serine proteases, structural features characteristic of previously described HtrA protein. In order to evaluate the biol. function(s) of Y. enterocolitica **HtrA**, an isogenic **mutant** was constructed by a reverse-genetics PCR-based approach.

Characterization of the **mutant** provided evidence supporting a stress response function for the Y. enterocolitica **htrA** gene product. In contrast to the parent strain, the mutant showed increased sensitivity to killing by H2O2, O2- and temp. stress (50.degree.). The mutant was avirulent in the murine **yersiniosis** infection model and offered partial protection to mice challenged with the parent strain. Further studies with the Y. enterocolitica **htrA mutant** should increase our knowledge of the host-pathogen interactions which occur during **Yersinia** infections.

L29 ANSWER 14 OF 28 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 96351471 MEDLINE
DOCUMENT NUMBER: 96351471 PubMed ID: 8717403
TITLE: Attenuated Salmonella as live oral vaccines against typhoid fever and as live vectors.
AUTHOR: Levine M M; Galen J; Barry E; Noriega F; **Chatfield S**; Sztein M; **Dougan G**; Tacket C
CORPORATE SOURCE: Center for Vaccine Development, University of Maryland School of Medicine, Baltimore 21201, USA.
SOURCE: JOURNAL OF BIOTECHNOLOGY, (1996 Jan 26) 44 (1-3) 193-6. Ref: 19
Journal code: AL6; 8411927. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961025
Last Updated on STN: 20000303
Entered Medline: 19961016

AB Attenuated Salmonella typhi vaccine strain CVD 908, which harbors deletion **mutations** in aroC and **aroD**, has been shown to be well-tolerated and highly immunogenic, eliciting impressive serum antibody, mucosal IgA and cell-mediated immune responses. A further derivative prepared by introducing a deletion in **htrA** (which encodes a heat-shock protein that also has activity as a serine protease in CVD 908 (Chatfield et al., unpublished data) resulted in CVD 908-**htrA**. In phase 1 clinical trials, CVD 908-**htrA** appears very attractive as a live oral vaccine candidate. Both CVD 908 and CVD 908-**htrA** are useful as live vector vaccines to deliver foreign antigens to the immune system. Conditions that enhance the expression and immunogenicity of foreign antigens carried by CVD 908 and CVD 908-**htrA** are being investigated.

L29 ANSWER 15 OF 28 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 94341908 MEDLINE
DOCUMENT NUMBER: 94341908 PubMed ID: 8063417
TITLE: Characterization of defined ompR mutants of Salmonella typhi: ompR is involved in the regulation of Vi polysaccharide expression.
AUTHOR: Pickard D; Li J; Roberts M; Maskell D; Hone D; Levine M; **Dougan G**; **Chatfield S**
CORPORATE SOURCE: Department of Biochemistry, Imperial College of

Science, Technology and Medicine, London, United Kingdom.
SOURCE: INFECTION AND IMMUNITY, (1994 Sep) 62 (9) 3984-93.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X78270
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941005
Last Updated on STN: 19941005
Entered Medline: 19940921

AB The ompB operon, comprising the ompR and envZ genes, was cloned from a Salmonella typhi Ty2 cosmid bank and characterized by DNA sequence analysis. The S. typhi ompR and envZ genes contained open reading frames encoding proteins of 240 and 451 amino acids, respectively. Comparison with the Salmonella typhimurium OmpB protein sequences revealed 99.5% homology. The DNA sequence data were used to identify appropriate restriction sites for generating a defined deletion of 517 bp within the open reading frame of the ompR gene. This deletion was introduced by homologous recombination into the chromosomes of two S. typhi strains which already harbored defined deletions in both the aroC and **aroD** genes. The presence of the deletions within ompR was confirmed by Southern hybridization and sequencing of the DNA fragments surrounding the deleted regions by PCR. The S. typhi ompR **mutants** displayed a marked decrease in OmpC and OmpF porin expression as demonstrated by examination of outer membrane preparations. It was also found that S. typhi strains harboring the defined ompR deletions no longer agglutinated with Vi antiserum. However, when a functional ompB operon was introduced back into the S. typhi ompR **mutants**, either on a multicopy plasmid or as a single-copy chromosomal replacement, the Vi+ phenotype was restored. The levels of Vi synthesis were also found to be sensitive to different concentrations of sodium chloride present in the growth medium, although the levels of sensitivity varied between different isolates of S. typhi. It is therefore concluded that the ompR-envZ two component regulatory system plays an important role in the regulation of Vi polysaccharide synthesis in S. typhi and that one of the environmental signals for this regulation may be osmolarity.

L29 ANSWER 16 OF 28 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 11
ACCESSION NUMBER: 1993:109672 CAPLUS
DOCUMENT NUMBER: 118:109672
TITLE: Attenuated **bacteria** expressing antigenic protein genes and their use as vaccines
INVENTOR(S): Charles, Ian George; **Chatfield, Steven Neville**; Fairweather, Neil Fraser
PATENT ASSIGNEE(S): Wellcome Foundation Ltd., UK
SOURCE: PCT Int. Appl., 23 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9215689	A1	19920917	WO 1992-GB387	19920305
W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US				
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
AU 9213508	A1	19921006	AU 1992-13508	19920305
AU 664360	B2	19951116		
EP 574466	A1	19931222	EP 1992-905914	19920305
EP 574466	B1	19990519		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL				
JP 06505158	T2	19940616	JP 1992-505563	19920305
HU 66833	A2	19950130	HU 1993-2492	19920305
HU 219535	B	20010528		
PL 170938	B1	19970228	PL 1992-296702	19920305
PL 171476	B1	19970530	PL 1992-312415	19920305
RU 2126447	C1	19990220	RU 1993-57957	19920305
CZ 285118	B6	19990512	CZ 1993-1005	19920305
AT 180280	E	19990615	AT 1992-905914	19920305
ES 2131069	T3	19990716	ES 1992-905914	19920305
NO 9302423	A	19930702	NO 1993-2423	19930702
US 5547664	A	19960820	US 1994-354776	19941212
US 5683700	A	19971104	US 1995-469507	19950606

PRIORITY APPLN. INFO.:		
GB 1991-4596	A	19910305
GB 1991-21208	A	19911004
WO 1992-GB387	A	19920305
US 1993-81361	B1	19930630
US 1994-246773	B1	19940520
US 1994-354776	A3	19941212

AB Attenuated **bacteria** contg. an antigenic protein gene fused to a promoter whose activity is induced by anaerobic conditions are described. These transformants can be used as vaccines. Salmonella typhimurium (aroA-aroD-) were transformed with a plasmid contg. the gene for tetanus toxin fragment C fused to the nirB promoter of Escherichia coli. These **bacteria** were effective single-dose oral vaccines against tetanus toxin challenge in mice.

L29 ANSWER 17 OF 28 MEDLINE
ACCESSION NUMBER: 92334130 MEDLINE
DOCUMENT NUMBER: 92334130 PubMed ID: 1630300
TITLE: Impaired resistance to infection does not increase the virulence of Salmonella htrA live vaccines for mice.
AUTHOR: Strahan K; **Chatfield S N**; Tite J; **Dougan G**; Hormaeche C E
CORPORATE SOURCE: Department of Pathology, Cambridge, U.K.
SOURCE: MICROBIAL PATHOGENESIS, (1992 Apr) 12 (4) 311-7. Journal code: MIC; 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920904
Last Updated on STN: 19970203
Entered Medline: 19920820

AB We have described a new class of live attenuated salmonella vaccines

harbouring lesions in **htrA**, a stress protein gene previously. The virulence and invasiveness of Salmonella **htrA mutants** was investigated in three models of increased susceptibility to Salmonella infection. These included BALB/c mice, either given sublethal whole body irradiation (350 R) or administered rabbit anti-TNF alpha antiserum, and (CBA/NfemaleXBALB/cmale)F1 male mice which express the xid sex-linked B cell defect of CBA/N mice and are more susceptible to salmonellae than female littermates. Salmonella typhimurium **htrA mutants** derived from virulent strains, C5046 (C5 **htrA**::TnphoA) and BRD726 (SL1344 delta **htrA**) were not more invasive in immunosuppressed mice than in normal controls in the three mouse models of defective immunity. The results indicate that susceptibility to S. typhimurium **htrA** vaccines derived from virulent parents is not enhanced by conditions of impaired resistance to infection.

L29 ANSWER 18 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:403305 BIOSIS
DOCUMENT NUMBER: PREV199345062130
TITLE: Salmonella genetics and vaccine development.
AUTHOR(S): **Chatfield, S. (1)**; Li, J. L. (1); **Sydenham, M. (1)**; Douce, G.; **Dougan, G.**
CORPORATE SOURCE: (1) Vaccine Res. Unit, Medeva Group Res., Dep. Biochemistry, Imperial Coll. Sci. Technol. and Med., Wolfson Lab., London SW7 2AY UK
SOURCE: Hormaeche, C. E. [Editor]; Penn, C. W. [Editor]; Smyth, C. J. [Editor]. Symposium of the Society for General Microbiology, (1992) Vol. 49, pp. 299-312. Symposium of the Society for General Microbiology; Molecular biology of bacterial infection: Current status and future perspectives.
Publisher: Cambridge University Press The Pitt Building, Trumpington Street, Cambridge CB2 1RP, England.
Meeting Info.: Meeting Dublin, Ireland September 1992
ISSN: 0081-1394. ISBN: 0-521-43298-7.
DOCUMENT TYPE: Article
LANGUAGE: English

L29 ANSWER 19 OF 28 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 93038550 MEDLINE
DOCUMENT NUMBER: 93038550 PubMed ID: 1329726
TITLE: Inducible overproduction of the Aspergillus nidulans pentafunctional AROM protein and the type-I and -II 3-dehydroquinases from Salmonella typhi and Mycobacterium tuberculosis.
AUTHOR: Moore J D; Lamb H K; Garbe T; Servos S; **Dougan G**; Charles I G; Hawkins A R
CORPORATE SOURCE: Department of Biochemistry and Genetics, University of Newcastle upon Tyne, U.K.
SOURCE: BIOCHEMICAL JOURNAL, (1992 Oct 1) 287 (Pt 1) 173-81.
Journal code: 9YO; 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19930122
Entered Medline: 19921110

AB The aroQ gene of Mycobacterium tuberculosis, encoding a type-II 3-dehydroquinase, and the **aroD** gene of Salmonella typhi, encoding a type-I 3-dehydroquinase, have been highly overexpressed in Escherichia coli using the powerful trc promoter contained within the expression vector pKK233-2. The M. tuberculosis type-II 3-dehydroquinase has been purified in bulk from overproducing strains of E. coli to greater than 95% homogeneity. The protein is extremely heat-stable, is active as a homododecamer and has the lowest reported Km value of any type-II 3-dehydroquinase. The pentafunctional aromA gene of Aspergillus nidulans has been overexpressed more than 120-fold in an A. nidulans aromA- qutB- double **mutant** from a truncated quinate-inducible qutE promoter, such that the AROM protein is visible as a significant fraction (approx. 6%) in cell-free crude extracts. The M. tuberculosis aroQ gene has been fused to the same truncated qutE promoter and shown to encode quinate-inducible 3-dehydroquinase activity that allows a qutE- **mutant** strain of A. nidulans to utilize quinate as sole carbon source.

L29 ANSWER 20 OF 28 MEDLINE
ACCESSION NUMBER: 92261298 MEDLINE
DOCUMENT NUMBER: 92261298 PubMed ID: 1584006
TITLE: Evaluation of Salmonella typhimurium strains harbouring defined **mutations** in **htrA** and aroA in the murine salmonellosis model.
AUTHOR: **Chatfield S N**; Strahan K; Pickard D; Charles I G; Hormaeche C E; **Dougan G**
CORPORATE SOURCE: Vaccines Research Unit, Medeva Group Research, Wellcome Research Labs, Beckenham, Kent, U.K.
SOURCE: MICROBIAL PATHOGENESIS, (1992 Feb) 12 (2) 145-51. Journal code: MIC; 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920626
Last Updated on STN: 19970203
Entered Medline: 19920618

AB Derivatives of the mouse-virulent Salmonella typhimurium strain SL1344 were constructed harbouring defined **mutations** in **htrA**, aroA or **htrA** aroA combined. When administered orally or intravenously to BALB/c mice, all the **mutants** were found to be highly attenuated. All **mutants** were able to confer significant protection against lethal challenge with SL1344 after a single oral dose of live organisms. SL1344 **htrA mutants** persisted in livers and spleens at a lower level than SL1344 aroA **mutants** after intravenous administration. SL1344 **htrA** aroA **mutants** persisted at an even lower level and were cleared from the livers and spleens of mice within 21 days of intravenous administration. Thus **htrA** and **htrA** aroA **mutants** can be considered as potential oral vaccines against

salmonellosis.

L29 ANSWER 21 OF 28 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1991-325215 [44] WPIDS
DOC. NO. CPI: C1991-140539
TITLE: Attenuated microorganism useful in live vaccines - attenuated by mutation in DNA sequence encoding e.g. a heat shock protein.
DERWENT CLASS: B04 D16
INVENTOR(S): CHARLES, I G; **CHATFIELD, S N**; **DOUGAN, G**; HORMAECHE, C E; JOHNSON, K S; HORMAECHE, C; CHARLES, I; JOHNSON, K; **CHATFIELD, S**
PATENT ASSIGNEE(S): (WELL) WELLCOME FOUND LTD; (GLAX) GLAXO WELLCOME INC
COUNTRY COUNT: 25
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9115572	A	19911017	(199144)*		26
RW: AT BE CH DE DK ES FR GB GR IT LU NL SE					
W: AU CA HU JP KR US					
AU 9175417	A	19911030	(199205)		
ZA 9102397	A	19921125	(199301)		23
EP 524205	A1	19930127	(199304)	EN	26
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE					
TW 205567	A	19930511	(199337)		
JP 05507842	W	19931111	(199350)		11
NZ 237616	A	19940325	(199426)		
HU 65496	T	19940628	(199429)		
AU 659995	B	19950608	(199531)		
EP 524205	B1	19970827	(199739)	EN	19
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE					
DE 69127440	E	19971002	(199745)		
ES 2106776	T3	19971116	(199801)		
US 5804194	A	19980908	(199843)		
IL 97720	A	19990620	(199937)		
US 5980907	A	19991109	(199954)		
PH 29735	A	19960517	(200012)		
HU 217776	B	20000428	(200030)		
JP 3054440	B2	20000619	(200033)		11
KR 202771	B1	19990615	(200061)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
ZA 9102397	A	ZA 1991-2397	19910328
EP 524205	A1	EP 1991-906493	19910328
		WO 1991-GB484	19910328
TW 205567	A	TW 1991-102452	19910328
JP 05507842	W	JP 1991-506347	19910328
		WO 1991-GB484	19910328
NZ 237616	A	NZ 1991-237616	19910328
HU 65496	T	WO 1991-GB484	19910328
		HU 1992-3098	19910328
AU 659995	B	AU 1991-75417	19910328

EP 524205	B1		EP 1991-906493	19910328
DE 69127440	E		WO 1991-GB484	19910328
			DE 1991-627440	19910328
			EP 1991-906493	19910328
ES 2106776	T3		WO 1991-GB484	19910328
US 5804194	A	Cont of	EP 1991-906493	19910328
		Cont of	WO 1991-GB484	19910328
		Cont of	US 1992-952737	19921130
			US 1994-239910	19940509
IL 97720	A		US 1994-350741	19941207
US 5980907	A	Cont of	IL 1991-97720	19910328
		Cont of	WO 1991-GB484	19910328
		Cont of	US 1992-952737	19921130
		Cont of	US 1994-239910	19940509
			US 1994-350741	19941207
PH 29735	A		US 1995-463875	19950605
HU 217776	B		PH 1991-42219	19910327
			WO 1991-GB484	19910328
JP 3054440	B2		HU 1992-3098	19910328
			JP 1991-506347	19910328
KR 202771	B1		WO 1991-GB484	19910328
			KR 1992-702407	19920930

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 524205	A1	Based on	WO 9115572
JP 05507842	W	Based on	WO 9115572
HU 65496	T	Based on	WO 9115572
AU 659995	B	Previous Publ.	AU 9175417
		Based on	WO 9115572
EP 524205	B1	Based on	WO 9115572
DE 69127440	E	Based on	EP 524205
		Based on	WO 9115572
ES 2106776	T3	Based on	EP 524205
US 5980907	A	Cont of	US 5804194
HU 217776	B	Previous Publ.	HU 65496
		Based on	WO 9115572
JP 3054440	B2	Previous Publ.	JP 05507842
		Based on	WO 9115572

PRIORITY APPLN. INFO: GB 1990-7194 19900330

AN 1991-325215 [44] WPIDS

AB WO 9115572 A UPAB: 20000105

Microorganisms (I) for use in immunoprophylaxis are attenuated as a result of the presence of a mutation in the DNA sequence of the microorganism which encodes, or which regulates the expression of DNA encoding, a protein that is produced in response to environmental stress. The microorganism is opt. capable of expression DNA encoding a heterologous antigen. Also new is a vaccine contg. (I).

The protein is a nutrient deprivation protein, toxic stress protein, metabolic distress protein or especially a heat shock protein encoded by the htrA gene. The microorganism is a **bacterium** such as **Bordetella, Vibrio,** Haemopilus, Esherichia or especially Salmonella.

USE/ADVANTAGE - (I) are useful in live vaccines and

immunoprophylaxis of e.g. salmonellosis, whooping cough, meningitis and gonorrhoea. Dosage of S. typti is 10 power 9 - 10 power 11 organisms/dose.

An attentuated form of S. typhimurium (strain 046) had log 10 ID50 of more than 9 cells, cf. the parental virulent strain C5 which had a log 10 LD50 of 6.38 cells, 28 days following oral administration.

Dwg.0/3

ABEQ ZA 9102397 A UPAB: 19930928

Microorganisms (I) for use in immunoprophylaxis are attenuated as a result of the presence of a mutation in the DNA sequence of the microorganism which encodes, or which regulates the expression of DNA encoding, a protein that is produced in response to environmental stress. The microorganism is opt. capable of expression DNA encoding a heterologous antigen. Also new is a vaccine contg. (I).

The protein is a nutrient deprivation protein, toxic stress protein, metabolic distress protein or esp. a heat shock protein encoded by the htrA gene. The microorganism is a **bacterium** e.g. **Bordetella**, **Vibrio**, Haemopilus, Esherichia or especially Salmonella.

USE/ADVANTAGE - (I) are useful in live vaccines and immunoprophylaxis of e.g. salmonellosis, whooping cough, meningitis and gonorrhoea. Dosage of S. typti is 10 power(9)- 10 power (11) organisms/dose.

An attenuated form of S. typhimurium (strain 046) and log 10 ID50 of more than 9 cells, cf. the parental virulent strain C5 which had a log 10LD50 of 6.38 cells, 28 days following oral administration

ABEQ EP 524205 B UPAB: 19970926

A vaccine comprising a pharmaceutically acceptable carrier and an effective amount of a **bacterium** attenuated by a non-reverting **mutation** in the **htrA** gene.

Dwg.0/3

L29 ANSWER 22 OF 28 MEDLINE DUPLICATE 13

ACCESSION NUMBER: 92101612 MEDLINE

DOCUMENT NUMBER: 92101612 PubMed ID: 1759503

TITLE: Construction of genetically defined double aro mutants of Salmonella typhi.

AUTHOR: Hone D M; Harris A M; **Chatfield S**; **Dougan G**; Levine M M

CORPORATE SOURCE: Department of Medicine, University of Maryland School of Medicine, University of Maryland, Baltimore 21201.

CONTRACT NUMBER: RO1-AI-29471 (NIAID)

SOURCE: VACCINE, (1991 Nov) 9 (11) 810-6.
Journal code: X6O; 8406899. ISSN: 0264-410X.

PUB. COUNTRY: ENGLAND: United Kingdom

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199202

ENTRY DATE: Entered STN: 19920223
Last Updated on STN: 19920223
Entered Medline: 19920206

AB The construction of genetically defined, double aro **mutant** strains CVD906 and CVD908, which were derived from Salmonella typhi strain ISP1820 (a recent isolate of S. typhi from Chile) and from

laboratory strain Ty2, respectively, is described. Strains CVD906 and CVD908 differ from previously described aro **mutants** of S. typhi as their aro deletion **mutations** do not extend beyond the limits of the **mutated** aro genes, and no antibiotic-resistance genes, plasmid sequences or S. typhimurium DNA sequences remain in the **mutant** strains. In minimal medium the aro **mutants** of S. typhi are unable to replicate whereas the wild type parent strains grow well in minimal medium. Using intraperitoneal inoculation of mice with S. typhi strains suspended in hog gastric mucin as a virulence assay, it is shown that the single aro **mutants** and the double aro **mutants** of Ty2 and ISP1820 are attenuated in mice. Trans complementation of the aro **mutants** with the aroC gene or **aroD** gene, or both, results in strains that are phenotypically identical to that of the wild type parents indicating that no measurable additional changes other than loss of the aro gene function occurred during strain construction.

L29 ANSWER 23 OF 28 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 91251770 MEDLINE
DOCUMENT NUMBER: 91251770 PubMed ID: 1645840
TITLE: The role of a stress-response protein in Salmonella typhimurium virulence.
AUTHOR: Johnson K; Charles I; **Dougan G**; Pickard D; O'Gaora P; Costa G; Ali T; Miller I; Hormaeche C
CORPORATE SOURCE: Department of Pathology, University of Cambridge, UK.
SOURCE: MOLECULAR MICROBIOLOGY, (1991 Feb) 5 (2) 401-7. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X54548
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 19910728
Last Updated on STN: 19990129
Entered Medline: 19910705

AB We recently described the use of selective transposon **mutagenesis** to generate a series of avirulent **mutants** of a pathogenic strain of Salmonella typhimurium. Cloning and sequencing of the insertion sites from two of these **mutants** reveals that both have identical locations within an open reading frame that is highly homologous to a gene, **htrA**, encoding a heat-shock protein in Escherichia coli. DNA sequence analysis of S. typhimurium **htrA** reveals the presence of a gene capable of encoding a protein with a calculated Mr of 49316 that has 88.7% protein:protein homology with its E. coli counterpart. In E. coli, lesions in this gene, also known as degP, reduce proteolytic degradation of aberrant periplasmic proteins. Characteristics of the S. typhimurium **htrA mutants**, 046 and 014, in vivo and in vitro suggested that they are avirulent because of impaired ability to survive and/or replicate in host tissues. In vitro, the S. typhimurium **htrA mutants** 046 and 014 are not temperature-sensitive but were found to be more susceptible to oxidative stress than the parent, suggesting that they may be less able to withstand oxidative killing within macrophages.

L29 ANSWER 24 OF 28 MEDLINE
ACCESSION NUMBER: 91259034 MEDLINE
DOCUMENT NUMBER: 91259034 PubMed ID: 2045778
TITLE: Molecular cloning and characterization of the aroD gene encoding 3-dehydroquinase from Salmonella typhi.
AUTHOR: Servos S; **Chatfield S**; Hone D; Levine M; Dimitriadis G; Pickard D; **Dougan G**; Fairweather N; Charles I
CORPORATE SOURCE: Department of Molecular Biology, Wellcome Biotech, Beckenham, Kent, UK.
SOURCE: JOURNAL OF GENERAL MICROBIOLOGY, (1991 Jan) 137 (Pt 1) 147-52.
Journal code: I87; 0375371. ISSN: 0022-1287.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M36026; GENBANK-M36027; GENBANK-M36028; GENBANK-M36029; GENBANK-M36030; GENBANK-M36031; GENBANK-M36032; GENBANK-M55527; GENBANK-M55528; GENBANK-X54546
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 19910802
Last Updated on STN: 19910802
Entered Medline: 19910717

AB The **aroD** gene from Salmonella typhi, encoding 5-dehydroquinate hydrolyase (3-dehydroquinase), has been cloned into Escherichia coli and the DNA sequence determined. The **aroD** gene was isolated from a cosmid gene bank by complementation of an S. typhimurium **aroD mutant**. Analysis of the DNA sequence revealed the presence of an open reading frame capable of encoding a protein of 252 amino acids with a calculated Mr of 27706. Comparison of the deduced S. typhi 3-dehydroquinase protein sequence with that elucidated for E. coli revealed 69% homology. Alignment of the S. typhi sequence and equivalent Aspergillus nidulans and Saccharomyces cerevisiae sequences showed that homology was lower, at 24%, but still significant. Use of a minicell expression system demonstrated that a polyclonal antibody raised against E. coli 3-dehydroquinase cross-related with its S. typhi counterpart.

L29 ANSWER 25 OF 28 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 91181301 MEDLINE
DOCUMENT NUMBER: 91181301 PubMed ID: 2008797
TITLE: Oral vaccination of calves against experimental salmonellosis using a double aro mutant of Salmonella typhimurium.
AUTHOR: Jones P W; **Dougan G**; Hayward C; Mackensie N; Collins P; **Chatfield S N**
CORPORATE SOURCE: Karolinska Institute, Dept. Clinical Bacteriology, Huddinge Hospital, Sweden.
SOURCE: VACCINE, (1991 Jan) 9 (1) 29-34.
Journal code: X6O; 8406899. ISSN: 0264-410X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910519

Last Updated on STN: 19910519
Entered Medline: 19910501

AB An attenuated derivative of the fully calf virulent Salmonella typhimurium strain ST4/74 was constructed by introducing stable **mutations** into genes aroA and **aroD** rendering the strain dependent for growth on aromatic compounds. The strain (BRD562) was used to vaccinate orally ten Friesian calves 7 days after birth with approximately 10(10) live organisms. The only effects of vaccination in the calves was a transient faecal excretion of the organism, mild transient diarrhoea and mild pyrexia in one animal. Two of these animals were tested for the presence of BRD562 at necropsy 56 days after birth but none were shown to be infected. Eight of the vaccinated calves were subjected to an oral challenge infection with ST4/74 28 days after vaccination and seven out of the eight animals survived. The only effect of challenge in the surviving calves was mild pyrexia and transient excretion of ST4/74 in their faeces; only one of the seven animals was found to be infected at necropsy 28 days after challenge. The eighth challenged calf scoured severely and was killed in extremis 7 days after challenge. Four unvaccinated control calves infected orally with ST4/74 28 days after birth all scoured severely and were euthanased for humane reasons 5-7 days later. This study suggests that vaccination with an attenuated strain of S. typhimurium harbouring **mutations** in two aro genes is a safe and effective way of protecting young calves against experimental S. typhimurium infection.

L29 ANSWER 26 OF 28 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1990-363446 [49] WPIDS
DOC. NO. CPI: C1990-157936
TITLE: Attenuated **bacteria** for use as live vaccines - have gene mutated, which regulates one or more genes concerned with outer membrane proteins esp. porin.
DERWENT CLASS: B04 C03 D16
INVENTOR(S): **CHATFIELD, S N**; DORMAN, C J; **DOUGAN, G**; HIGGINS, C F; CHARFIELD, S N
PATENT ASSIGNEE(S): (LIST-N) LISTER INST PREVENTIVE MEDICINE; (ROYA-N) ROYAL SOC; (UYDU-N) UNIV DUNDEE; (WELL) WELLCOME FOUND LTD; (LIST-N) LISTER INST PREVENTIVE M; (GLAX) GLAXO WELLCOME INC
COUNTRY COUNT: 16
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 400958	A	19901205	(199049)*		9
R: AT BE CH DE ES FR GB GR IT LI LU NL SE					
JP 03117481	A	19910520	(199126)		
EP 400958	B1	19950913	(199541)	EN	12
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE					
DE 69022290	E	19951019	(199547)		
ES 2077028	T3	19951116	(199551)		
US 5527529	A	19960618	(199630)		7
US 5851519	A	19981222	(199907)		
JP 3024982	B2	20000327	(200020)		7

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
EP 400958	A		EP 1990-305819	19900529
JP 03117481	A		JP 1990-139566	19900529
EP 400958	B1		EP 1990-305819	19900529
DE 69022290	E		DE 1990-622290	19900529
			EP 1990-305819	19900529
ES 2077028	T3		EP 1990-305819	19900529
US 5527529	A	Cont of	US 1990-528972	19900529
		Cont of	US 1992-827584	19920127
			US 1995-419741	19950410
US 5851519	A	Cont of	US 1990-528972	19900529
		Div ex	US 1992-827584	19920127
			US 1995-419618	19950410
JP 3024982	B2		JP 1990-139566	19900529

FILING DETAILS:

PATENT NO	KIND		PATENT NO
DE 69022290	E	Based on	EP 400958
ES 2077028	T3	Based on	EP 400958
JP 3024982	B2	Previous Publ.	JP 03117481

PRIORITY APPLN. INFO: GB 1989-12330 19890530

AN 1990-363446 [49] WPIDS

AB EP 400958 A UPAB: 19970417

An attenuated **bacteria**, with a **mutation** in a gene concerned with regulating one or more additional genes, is new. The genes regulated encode an outer membrane protein and are porin genes. The regulating gene is Omp. R. The **bacteria** is gram negative and selected from Salmonella, **Bordetella**, Viloris, **Haemophilus** and Escherichia genera, pref. it is from S. typhi an A-, omp-, S. typhimium omp-, or aroA-, omp R- or S. dublin omp R- or aroA, ompR-. Opt. a second gene is also **mutated**, which encodes an enzyme involved in an essential auxotrophic pathway. This gene is pref. anoA, aroC, or **aroD** .

USE/ADVANTAGE - **Bacteria** attenuated in such a way that can be used as live vaccines in human and animal medicine. It can be used in a prophylactic treatment of a **bacterial** infection, in an effective dose which depends on various clinical factors. For S.typhi a dosage of 109-1011 organisms/dose is used for a 70 kg human patient. @(9pp Dwg.No.0/1)@

ABEQ EP 400958 B UPAB: 19951019

A vaccine formulation comprising a **bacterium** attenuated by a non-revering mutation in the ompR gene in admixture with a pharmaceutically acceptable excipient.

Dwg.0/0

ABEQ US 5527529 A UPAB: 19960731

A pharmaceutical composition for oral administration to a subject for inducing immunity to a pathogenic Salmonella **bacterium** , which composition comprises a pharmaceutically acceptable excipient and an attenuation form of said Salmonella **bacterium**, the attenuation being attributable to a non-reverting mutation in the ompR gene of said Salmonella **bacterium**.

Dwg.0/1

L29 ANSWER 27 OF 28 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:84145 CAPLUS
DOCUMENT NUMBER: 112:84145
TITLE: Live vaccines containing attenuated microorganisms having double mutations in genes in the aromatic biosynthetic pathway
INVENTOR(S): **Dougan, Gordon; Chatfield, Steven Neville**
PATENT ASSIGNEE(S): Wellcome Foundation Ltd., UK
SOURCE: Eur. Pat. Appl., 13 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 322237	A1	19890628	EP 1988-312203	19881222
EP 322237	B1	19940323		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
WO 8905856	A1	19890629	WO 1988-GB1143	19881222
W: AU, BR, DK, HU, JP, SU, US				
AU 8929193	A1	19890719	AU 1989-29193	19881222
AU 619519	B2	19920130		
BR 8807376	A	19900320	BR 1988-7376	19881222
ZA 8809605	A	19900829	ZA 1988-9605	19881222
JP 02502785	T2	19900906	JP 1989-501160	19881222
HU 55242	A2	19910528	HU 1989-702	19881222
HU 216449	B	19990628		
CA 1327331	A1	19940301	CA 1988-586868	19881222
AT 103331	E	19940415	AT 1988-312203	19881222
ES 2061700	T3	19941216	ES 1988-312203	19881222
IL 88766	A1	19950731	IL 1988-88766	19881222
KR 9710759	B1	19970630	KR 1988-17199	19881222
RU 2114172	C1	19980627	RU 1988-4742131	19881222
DK 8904126	A	19890822	DK 1989-4126	19890822
US 5811105	A	19980922	US 1995-449297	19950524
US 5770214	A	19980623	US 1995-484314	19950607

PRIORITY APPLN. INFO.:

GB 1987-30037	A	19871223	
EP 1988-312203	A	19881222	
WO 1988-GB1143	A	19881222	
US 1989-399539	B1	19890822	
US 1991-642138	B1	19910115	
US 1992-857092	B1	19920320	
US 1992-979460	B1	19921120	
US 1993-135436	B3	19931013	

AB An attenuated microorganism harboring 2 mutated genes, each of which is located in the organism's arom. biosynthetic pathway is useful as a vaccine. The attenuated microorganism can be genetically engineered so as to express antigens from other pathogens, thus making a range of multivalent vaccines. Salmonella typhimurium aroA aroC double mutant was prepd. by transposon mutagenesis. Balb/c mice treated by oral administration of 109-1010 of the mutant resisted oral challenge by the parental virulent strain (SL 1344) of S. typhimurium 28 and 70 days post immunization. Oral tablets

contained freeze-dried S. typhi double mutant 70.0, Aerosil-200 0.5, Dipac 235.0, crosslinked Povidone 7.0, microcryst. cellulose, 35.0, and Mg stearate 2.5 mg coated with Opadry Enteric OY-P-7156 35.0 mg.

L29 ANSWER 28 OF 28 MEDLINE

ACCESSION NUMBER:	89218937 MEDLINE
DOCUMENT NUMBER:	89218937 PubMed ID: 2523513
TITLE:	Bacteriophage P22 as a vehicle for transducing cosmid gene banks between smooth strains of Salmonella typhimurium: use in identifying a role for aroD in attenuating virulent Salmonella strains.
AUTHOR:	Miller I A; **Chatfield S; Dougan G** ; Desilva L; Joysey H S; Hormaeche C
CORPORATE SOURCE:	Department of Pathology, University of Cambridge, UK.
SOURCE:	MOLECULAR AND GENERAL GENETICS, (1989 Jan) 215 (2) 312-6. Journal code: NGP; 0125036. ISSN: 0026-8925.
PUB. COUNTRY:	GERMANY, WEST: Germany, Federal Republic of
	Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:	English
FILE SEGMENT:	Priority Journals
ENTRY MONTH:	198906
ENTRY DATE:	Entered STN: 19900306 Last Updated on STN: 19900306 Entered Medline: 19890608

AB A cosmid gene bank of the virulent Salmonella typhimurium C5 was constructed in Escherichia coli K12. The bank was repackaged into bacteriophage heads and transduced into the semi-rough S. typhimurium strain AS68 which expresses the LamB lambda receptor protein. Approximately 6000 ampicillin-resistant transductants were pooled and used as host for the propagation of bacteriophage P22. The P22 lysate was able to transduce cosmid recombinants to smooth strains of S. typhimurium and individual transductants were selected which complemented various S. typhimurium auxotrophic **mutations**. A stable **mutation** was introduced into the **aroD** gene of S. typhimurium C5. The resulting **aroD- mutant**, named CU038, was highly attenuated compared with the wild-type parent strain and BALB/c mice immunised orally with CU038 were well protected against challenge with the virulent C5 parental strain. Using the cosmid bank repackaged into bacteriophage P22 heads it was possible to isolate cosmid recombinants that could complement the **aroD mutation** of CU038 either by in vitro selection using minimal medium or in vivo selection for restoration of virulence in BALB/c mice. Repackaged P22 cosmid banks could provide a simple system for selecting in vivo for Salmonella virulence determinants. A Salmonella typhi strain harbouring **mutations** in aroA and **aroD** was constructed for potential use as a live oral typhoid vaccine in humans.

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FILE 'HOME' ENTERED AT 13:02:30 ON 06 FEB 2002

ER 5 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AN 1996:26382767 BIOTECHNO
TI An in vivo pathway for disulfide bond isomerization in Escherichia coli
AU Rietsch A.; Belin D.; Martin N.; Beckwith J.
CS Microbiology/Molec. Genetics Dept., Harvard Medical School, 200 Longwood Avenue,Boston, MA 02115, United States.
SO Proceedings of the National Academy of Sciences of the United States of America, (1996), 93/23 (13048-13053)
CODEN: PNASA6 ISSN: 0027-8424
DT Journal; Conference Article
CY United States
LA English
SL English

L29 ANSWER 6 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AN 1995:25353054 BIOTECHNO
TI Characterization of an Escherichia coli rotA mutant, affected in periplasmic peptidyl-prolyl cis/trans isomerase
AU Kleerebezem M.; Heutink M.; Tommassen J.
CS Department of Molecular Cell Biology, Utrecht University, Padualaan 8,3584 CH Utrecht, Netherlands.
SO Molecular Microbiology, (1995), 18/2 (313-320)
CODEN: MOMIEE ISSN: 0950-382X
DT Journal; Article
CY United Kingdom
LA English
SL English

L29 ANSWER 7 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AN 1993:24021018 BIOTECHNO
TI The activity of .sigma.(E), an Escherichia coli heat-inducible .sigma.-factor, is modulated by expression of outer membrane proteins
AU Mecsas J.; Rouviere P.E.; Erickson J.W.; Donohue T.J.; Gross C.A.
CS Department of Oral Biology, University of California,San Francisco, CA 94143, United States.
SO Genes and Development, (1993), 7/12 B (2618-2628)
CODEN: GEDEEP ISSN: 0890-9369
DT Journal; Article
CY United States
LA English
SL English

L29 ANSWER 8 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AN 1993:23053946 BIOTECHNO
TI A pathway for disulfide bond formation in vivo
AU Bardwell J.C.A.; Lee J.-O.; Jander G.; Martin N.; Belin D.; Beckwith J.
CS Microbiol./Molecular Genetics Dept., Harvard Medical School,Boston, MA 02115, United States.
SO Proceedings of the National Academy of Sciences of the United States of America, (1993), 90/3 (1038-1042)
CODEN: PNASA6 ISSN: 0027-8424
DT Journal; Article
CY United States
LA English
SL English

L29 ANSWER 9 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AN 1992:22339530 BIOTECHNO
TI A **periplasmic protein** disulfide oxidoreductase is required for transformation of Haemophilus influenzae Rd
AU Tomb J.-F.
CS Department of Molecular Biology, Johns Hopkins School of Medicine, PCTB 505, 725 North Wolfe Street,Baltimore, MD 21205, United States.
SO Proceedings of the National Academy of Sciences of the United States of

America, (1992), 89/21 (10252-10256)
CODEN: PNASA6 ISSN: 0027-8424
DT Journal; Article
CY United States
LA English
SL English

L29 ANSWER 10 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AN 1992:22236421 BIOTECHNO
TI A homologue of the Escherichia coli DsbA protein involved in disulphide bond formation is required for enterotoxin biogenesis in Vibrio cholerae
AU Yu J.; Webb H.; Hirst T.R.
CS The Biological Laboratory, University of Kent,Canterbury, Kent CT2 7NJ, United Kingdom.
SO Molecular Microbiology, (1992), 6/14 (1949-1958)
CODEN: MOMIEE ISSN: 0950-382X
DT Journal; Article
CY United Kingdom
LA English
SL English

L29 ANSWER 11 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AN 1992:22043886 BIOTECHNO
TI Identification and characterization of an Escherichia coli gene required for the formation of correctly folded alkaline phosphatase, a periplasmic enzyme
AU Kamitani S.; Akiyama Y.; Ito K.
CS Department of Cell Biology, Institute for Virus Research, Kyoto University,Kyoto 606-01, Japan.
SO EMBO Journal, (1992), 11/1 (57-62)
CODEN: EMJODG ISSN: 0261-4189
DT Journal; Article
CY United Kingdom
LA English
SL English

L29 ANSWER 12 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AN 1991:21336150 BIOTECHNO
TI Identification of a protein required for disulfide bond formation in vivo
AU Bardwell J.C.A.; McGovern K.; Beckwith J.
CS Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, United States.
SO Cell, (1991), 67/3 (581-589)
CODEN: CELLB5 ISSN: 0092-8674
DT Journal; Article
CY United States
LA English
SL English

=> d kwic 12

L29 ANSWER 12 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.
AB We describe a **mutation** (dsbA) that renders Escherichia coli severely defective in disulfide bond formation. In dsbA mutant cells, pulse-labeled .beta.-lactamase, alkaline phosphatase, and OmpA are secreted but largely lack disulfide bonds. These disulfideless proteins may represent in vivo **folding** intermediates, since they are protease sensitive and chase slowly into stable oxidized forms. The dsbA gene codes for a 21,000 M(r) **periplasmic protein** containing the sequence cys-pro-his-cys, which resembles the active sites of certain disulfide oxidoreductases. The purified DsbA protein is capable of. . .
CT *oxidoreductase; *bacterial **mutation**; *disulfide bond; *dna

sequence; *escherichia coli; *molecular cloning; *protein purification; article; nonhuman; priority journal

=>

ANSWER 4 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 1998:390126 BIOSIS
DN PREV199800390126
TI A new heat-shock gene, ppiD, encodes a peptidyl-prolyl isomerase required for **folding** of outer membrane proteins in Escherichia coli.
AU Dartigalongue, Claire; Raina, Satish (1)
CS (1) Dep. Biochim. Med., Cent. Med. Univ., 1 rue Michel-Servet, 1211 Geneve 4 Switzerland
SO EMBO (European Molecular Biology Organization) Journal, (July 15, 1998) Vol. 17, No. 14, pp. 3968-3980.
ISSN: 0261-4189.
DT Article
LA English

. . D. Pickard, and G. Dougan, Infect. Immun. 57:2758-2763, 1989) was characterized, and the transposon was shown to be inserted in **surA** , a gene which encodes a peptidylprolyl-cis,trans-isomerase. A defined **surA** deletion **mutation** was introduced into S. enterica serovar Typhimurium C5 and the mutant strain, named S. enterica serovar Typhimurium BRD1115, was extensively. . . BRD1115 was attenuated by at least 3 log units when administered orally or intravenously to BALB/c mice. Complementation of the **mutation** with a plasmid carrying the intact **surA** gene almost completely restored the virulence of BRD1115. In addition, S. enterica serovar Typhimurium BRD1115 demonstrated potential as a **vaccine** candidate, since mice immunized with BRD1115 were protected against subsequent challenge with S. enterica serovar Typhimurium C5. S. enterica serovar. . .

L3 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2000:173992 BIOSIS
DN PREV200000173992
TI Salmonella enterica serovar Typhimurium **surA** mutants are attenuated and effective live oral **vaccines**.
AU Sydenham, Mark; Douce, Gillian; Bowe, Frances; Ahmed, Saddif; Chatfield, Steve; Dougan, Gordon (1)
CS (1) Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AZ UK
SO Infection and Immunity., (March, 2000) Vol. 68, No. 3, pp. 1109-1115. ISSN: 0019-9567.
DT Article
LA English
SL English